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ZOOPLANKTON DISTRIBUTION AND ISOTOPE TURNOVER DURING OPERATION SWORDFISH

FINAL REPORT PART I

Contract AT(30-1)-3145

February 1965

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Woods Hole Oceanographic Institution

Woods Hole, Massachusetts

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Approved for Distribution

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INTRODUCTION

A major question in marine ecology is the contribution of zooplankton to various elemental cycles. One would like to know the flow rates of important elements that result from the feeding, growth, excretion, reproduction, and death of the many important planktonic species. There have been a few studies such as those of Conover (1961), Marshall and Orr (1961), and Pomeroy, Mathews and Min (1963) on phosphorus cycling, but the cycling of most physiologically important elements in most marine zooplankton species is completely unknown.

The turnover rates of various elements by zooplankton are also important from a practical viewpoint. Ketchum and Bowen (1958) discussed the question of vertical transport of radioisotopes in the sea caused by diurnal migrations of animals. They used the simplifying assumption that animals reach equilibrium for an isotope during each period spent in the water layers above and below the thermocline through which they migrate. Using reasonable values for the concentration factors (the ratio of concentration of an element in an organism to that in the water), the vertical range of migration, the period of the migration, and the mean population density of animals, they then predicted that biological transport might often equal or exceed that caused by vertical eddy diffusion. They

recognized, however, that present data on equilibrium times for marine plankton are scanty, making studies of rates of elemental uptake and exchange necessary.

Unfortunately the important oceanic zooplankton species have not been successfully cultured in the laboratory. Many have been kept alive for weeks and have grown and developed, but they often fail to complete their development or to reproduce, and the investigator seldom has the assurance that his animals are as healthy as they were in nature. For some purposes it should be possible to avoid the problems of long-term laboratory cultivation of zooplankton by collecting the animals at sea and immediately measuring important physiological parameters.

The present report describes the measurement of elimination rates of metabolically important elements by several zooplankton species, and measurement of the vertical migration of these same species to evaluate their contribution to vertical transport of elements. Several members of the staff of the Woods Hole Oceanographic Institution took advantage of the massive addition of radioisotopes into the Pacific Ocean afforded by Project SWORDFISH of the U.S. Atomic Energy Commission in 1962 to study basic problems in oceanography. Unfortunately, we received only short notice of this opportunity and were therefore severely limited in the amount of prior testing of our procedures. The USCGS ship PIONEER was furnished for our use and we were able to collect zooplankton that contained moderate levels of radioactivity following the nuclear test. A second series of experiments was performed aboard NSF ship ANTON BRUUN in the Indian Ocean in January-March, 1963; these differed from the PIONEER experiments principally in that the animals were labeled with radioisotopes following their capture. Basically the plan was to

measure the amount of radioactivity in the tissues and in particulate and soluble excreta. From these we learned both the state in which various elements are eliminated and the rates of turnover. The isotopes investigated were I^{131}, $Fe^{55,59}$, $Co^{58,60}$, Mn^{54}, Zn^{65}, $Sr^{89,90}$, and Ba^{140}, but significant data were obtained on only the first five elements. The animals studied included Pteropoda, Pyrosomatidae, Euphausiidae, Copepoda, Chaetognatha, and Salpidae.

The physical and chemical oceanographic measurements of the test site and the report of radioisotope distribution following the test will be given in separate reports. General features of the area will be given now, however, to aid in interpreting the present data. The sea temperature usually remained constant (15° to 18°C.) from the surface down to about 100 m. The main part of the thermocline was between 100 and 300 m, the temperature dropping to about 8° at 300 m. The temperature then slowly decreased with depth, becoming 4°C or less at 1200 m. Preliminary observation indicates that the surface currents generally set towards the south or east. However, more important for the present discussion, is the density discontinuity at the thermocline which sharply separated the upper 100 m layer from the deeper layers. The temperatures, salinities, and phosphate concentrations showed that the upper 100 m layer was well mixed and that it differed from deeper layers (Ketchum, Corwin, and Stimson 1964). It was an independent water mass and drifted over the deeper layers, creating a shear zone at the thermocline.

The test resulted in radioactive matter being added to the surface layer. This surface water drifted and mixed with outlying water, causing dilution; it also passed over the deeper layers. The shear between the surface and deeper layers resulted in contaminated water passing over

unlabeled, deep-dwelling zooplankton that subsequently ascended into it in their nightly migrations; it also left behind some radioactive individuals in deeper layers when the contaminated water moved on.

ACKNOWLEDGEMENTS

I am grateful to the officers and crews of PIONEER and ANTON BRUUN for their cooperation in making possible the shipboard collections and experiments. Aboard PIONEER, Mr. Gordon K. Riel helped our search for radioactive plankton by tracing the position of labeled water with his *in situ* gamma scintillation spectrometer probe. Dr. George D. Grice and Dr. Michael M. Mullin kindly assisted with collecting, sorting, and identifying zooplankton at sea. Sincere appreciation is extended to the several zooplankton specialists mentioned later for identifying specimens. Dr. Victor E. Noshkin and Mr. Neil R. Andersen were very helpful in recommending procedures for chemical separations and for counting some of the samples, and Dr. Vaughan T. Bowen gave many helpful suggestions in almost all phases of this work. Finally, I must express my thanks to Mr. James P. Perras and Mrs. Marcia Rounbehler who carefully performed the major portion of the chemical separations and radioactive counting of samples from the PIONEER experiments.

METHODS

The methods employed for samples aboard PIONEER are divided into three phases. First, diurnal migration of important species was determined from vertical hauls with a closing net. Secondly, labeled zooplankton was collected, sorted, placed into clean water, and the radioactive

excretory products preserved aboard ship. Finally, ashore the radioactive elements in the organisms and the excretory products were separated and counted.

I. Diurnal Plankton Migrations.

The extent of vertical migration of various zooplankton species was determined from the number of individuals found in hauls taken at different depths throughout the day and night. Samples were taken from 100-0 m, 300-100 m, and 500-300 m at 13 stations in a 120 mile square area in the eastern Pacific Ocean near the test site. Each sample was a vertical haul of a 0.75 m or 0.5 m diameter closing net of No. 6 mesh (0.24 mm pores). The volume of water sampled was calculated as $\pi r^2 l$, where r is the radius of the mouth of the net and l is the length of the vertical haul. The contents of the net were preserved in jars with sea water containing 5% formalin (borax buffered). Ashore the animals were sorted into major groups. We identified and enumerated individuals of several groups assisted by the following: Dr. George Grice, euphausiids and pteropods; Dr. Charles E. Cuttress, ctenophores; Dr. J. L. Yount, salps and pyrosomas. The identifications and counting of other important groups were done entirely by these specialists: Mr. Paul N. Sund, chaetognaths; Dr. Elbert H. Ahlstrom, fishes; Dr. Abraham Fleminger, copepods. The specialists listed above also identified most of the radioactive animals used for the excretion experiments described below, except that Dr. George Grice identified the copepods.

II. Elimination Rates

The operations aboard PIONEER consisted of five steps: (1) collection of clean sea water prior to the test; (2) collection of zooplankton; (3) sorting and washing of plankton; (4) maintenance during excretion; and (5) separation and preservation of the animals and their excreta.

The excretion experiments fundamentally consisted of placing healthy, radioactive animals into uncontaminated sea water for a period of time, then removing them and measuring the radioactivity present in the water. The uncontaminated sea water was collected from the surface in a clean polyethylene bucket several days prior to the test and stored in several 20-l polyethylene carboys. One 20-l carboy with a stopcock near the bottom was kept in a refrigerator with its thermostat set to maintain surface sea water temperature. Water drawn from this carboy was nearly the same as that from which the animals were taken, except that it was free of recent radioactivity.

A few words are in order concerning collection of samples from the deck of a ship in a contaminated pool of water. Personal protection from accidental splashing was effected by foul-weather gear parka and pants, rubber boots, and rubber or polyethylene gloves. Openings at the wrists and ankles were taped shut. When finished on deck, the worker rinsed himself off with clean water from a hose on deck and went to a decontamination and changing room. Here he was inspected with a survey meter and could further scrub himself if necessary. When satisfactorily decontaminated he removed his boots, gloves, and foul-weather gear, and left them in the decontamination room. By restricting the number of people on deck, and requiring them to be checked and decontaminated before returning to other areas of the ship, gross contamination of other working areas was prevented.

Excretion experiments began a few days after the atomic test and continued for the next eleven days. Labelled zooplankton was collected in a 1-m diameter No. 6 net towed for 15 to 60 minutes as the ship drifted over the contaminated area. To avoid damaging the animals, the

tows lasted only long enough to get a sufficient number of individuals for the experiments. Most collections were made late at night at about 25 m depth; two, however, were made at shallower depths, and one dawn tow was made at 150 m and another at 300 m. The surface tows at night were considered most valuable for our purposes for several reasons. These collections contained the species that undergo diurnal vertical migrations. Secondly, the animals would not experience the rapid temperature and pressure changes to which they would have been subjected had they been brought from below the thermocline. Finally, these animals were probably in the contaminated water for several hours before their capture and had accumulated considerable amounts of radioactivity. A glass jar or polyethylene bag inside a metal cup at the cod end of the net assured that at least 500 ml of sea water was present with the animals when the net was hauled out. As soon as the net came aboard, the jar was removed and the catch immediately poured into about 5 l of the uncontaminated sea water (at 14°-16°C, the sea surface temperature) in a polyethylene bucket. The bucket was taken without delay to the laboratory (air temperature controlled at 18°C.) and the plankton transferred to a white enameled tray. From this time until the catch was sorted the captain headed the ship into the wind to minimize rolling and the accompanying splashing of the tray contents. The animals were separated into lots containing single species, as nearly as could be determined. First, the largest animals were picked out, using stainless-steel or bone spoons, and transferred to glass bowls containing uncontaminated sea water. When a suitable number had been obtained they were serially transferred through at least three washes. Each wash was sufficient to afford at least a ten fold dilution of the prior solution, ultimately giving a dilution factor of at least 10^3,

usually more than 10^4. Smaller animals were transferred to glass bowls by means of large-bore pipets with rubber bulbs and washed by repeated changes of the water to give a dilution of the initial radioactive contamination of 10^3 or more. Each group of animals was then placed in 400-500 ml of the uncontaminated sea water, also at 14°-16°C., in a polyethylene bag. The bag was hung inside a one or two liter polyethylene box, to catch possible leakage, and the box was placed in the 14°-16° refrigerator without lights. Polyethylene is permeable to oxygen, and previous experiments on board showed that many zooplankton species survive for at least several days under this treatment.

After several hours the bags were returned to the laboratory where the animals were removed and placed in 10% neutral formalin. Smaller animals such as copepods were removed directly by filtering through a 0.8μ membrane filter (Millipore®; hereafter designated MF); the animals and the filters were placed into the same vial of formaldehyde. The water from experiments on larger animals was similarly filtered and the filter preserved with the animals. Experiments were discarded if the animals appeared dead or unhealthy following their excretion period in the polyethylene bag.

Three kinds of ion exchange resins were used: a Strongly acidic Cation Exchanger (Dowex 50 W-12), a Strongly basic Anion Exchanger (Bio Rad Ag1-X10), and a Weakly basic Anion Exchanger (Bio Rad Ag 3-X4), hereafter designated SCE, SAE, and WAE, respectively. Ion exchange columns were prepared prior to the cruise. The resins were washed repeatedly with 10% HCl and distilled water, and 13-15 ml of each resin were slurried into a 15 mm (inside diameter) polyethylene tube; each column finally contained a series of beds: strong cation, strong anion, and weak anion exchanger

at the top, middle, and bottom, respectively. Each bed was about 50 mm deep and was separated from the others by a layer of glass wool. Glass wool plugs also supported the resins at the top and bottom of each column. The columns were equilibrated with 300 ml of clean, filtered sea water, sealed at both ends, and packed for the cruise.

The filtrate from each excretion experiment was passed through one of these triple ion exchange columns at 3 to 4 ml per minute and the effluent was saved in a 500 ml polyethylene bottle. After all of the filtrate had passed through, the columns were resealed and packed for shipment to the shore laboratory. The radioactivity present in the zooplankton when captured was thus separated into the following categories: (1) the preserved animals, containing whatever radioisotopes they had not excreted; (2) the filter, with the feces or other particulate excreta; (3) the three different ion exchange resins, each now loaded with those elements in an ionic state capable of exchanging; and (4) the effluent, containing soluble non-ionic or complexed radioisotopes. The resins and effluents from the initial, shipboard work will hereafter have the subscript one (i.e., SCE_1 or $Effluent_1$) to distinguish them from resins and effluents of the separation and purification phases of the work.

III. Separation and Counting of Radioisotopes

General. The first steps prior to analyzing samples for radioactivity were identification, enumeration, and weighing (damp dried and oven dried) of the animals used in the excretion experiments. A known amount of carrier, usually about 10 mg, of each element of interest was then added to each sample so that we could determine the effectiveness of our recovery and make corrections for losses. The separation of each isotope from the various samples is detailed below. Finally, the measured radioactivity

attributable to each isotope was corrected for decay to the same date so that all samples are comparable for each element.

Counting Equipment. The following techniques were used for counting gross gamma radioactivity of the whole, preserved animals, and the radioactivity of samples following separation, purification and weighing of each element.

A. Gross gamma and I^{131} gamma rays were counted with a thallium-activated NaI scintillation crystal and a Nuclear Chicago Model 186A decade scaler. A broad band of gamma energies was detected; the high background (about 240 cpm)prevented obtaining significant counts on many samples.

B. Other gamma emitters (Fe^{59}, $Co^{56,58,60}$, Zn^{65}, Mn^{54}) were detected and counted as above except that a Nuclear Chicago Model 1810 single-channel differential pulse hight analyzer was interposed to select only the gamma peaks of the isotope in question. This lowered the background by a factor of about 100 and also discriminated against some possible contaminants. A Nuclear Chicago Model C120-1 automatic sample changer was used and all samples were counted several times to guard against possible erroneous counts or print-outs.

C. The weak x-rays of Fe^{55} were counted with a Nuclear Chicago Model D47 windowless detector, proportional gas (90% argon, 10% methane), and a Model 181A decade scaler and Model C110-B automatic sample changer. A 1 mv sensitivity gave a plateau of 1 to 3.5%; detector efficiency was about 8%.

Counting Statistics and Calculations. Standard deviation, s, both for samples and for background, was calculated as:

$$s = \frac{cpm}{\sqrt{n}} \qquad (1)$$

where n is total number of counts. Standard deviation of the net count

rate (sample rate minus background rate), s_3, was:

$$s_3 = \sqrt{s_1^2 + s_2^2} \qquad (2)$$

where s_1 and s_2 are standard deviations of sample and of background. If the net count rate of a sample was less than twice s_3, the activity was not considered significant.

After measuring the radioactivity of an isotope in all samples from an experiment, the radioactivity in the animal and the excretory products was summed. It was considered that this mount of activity was originally entirely in the animal and that the amounts found on the filter, on the three ion exchange resins, and in the effluent were eliminated by the animal during the experiment. The activities found on the filter, on the three resins, and in the effluent were converted to percentages of the total; the elimination coefficient, λ, was calculated from:

$$\lambda = \frac{\ln (1.0 - \% \text{ excreted})}{\Delta T} \qquad (3)$$

where ΔT is the duration of elimination period in hours. All of the elimination rates are therefore directly comparable to each other.

Separation of Iodine-131. Because of the 8-day half-life of this isotope, analyses were begun as soon as the samples arrived at the laboratory. Even so, only the tissues, the SAE_1, and the $Effluent_1$ could be done before the samples decayed to undetectable levels. The preliminary steps in separating I^{131} from these three fractions differed, but the final steps of purification followed Procedure 15 of Kleinberg and Cowan (1960).

The I^{131} was separated from tissues by driving it off as HI (and some I_2) and catching it in NaOH as follows: (1) Place dry, weighed animals in 125-ml Erlenmeyer flask. Add dried residue of formaldehyde preservative (if more than one species had inadvertantly been used in an experiment, each species and the formaldehyde was analyzed separately).

(2) Add 1 ml 0.21 $\underline{\underline{N}}$ KIO_3 carrier, 2 ml 1 $\underline{\underline{M}}$ $NaHSO_3$, and 4 ml 36 $\underline{\underline{N}}$ H_2SO_4. Attach slender glass tubing to side arm above flask (Figure 1). (3) Insert tubing into 100-ml burette containing 40 ml 1 $\underline{\underline{N}}$ NaOH. (4) Heat flask slowly, flushing vapors through NaOH trap with slow stream of N_2, until H_2SO_4 fumes appear in flask; hold at that temperature 10 minutes. (5) Drain burette and rinse with distilled water into flask; add rinsings from bubbler tube. (6) Neutralize NaOH with 3 ml 16 $\underline{\underline{N}}$ HNO_3 and add 0.2 ml 1 $\underline{\underline{M}}$ $NaHSO_3$ to reduce any I_2 that might have fumed over. (7) Transfer solution to separatory funnel and oxidize I^- to I_2 by dropwise addition of 1 $\underline{\underline{N}}$ $NaNO_2$. (8) When brown solution persists, extract I_2 into 10 ml CCl_4 and withdraw into another separatory funnel. Add 5 drops $NaNO_2$ to aqueous fraction and perform another CCl_4 extraction. Combine CCl_4 fractions for the final steps of purification given below.

The charred remains of tissues were oxidized by repeatedly heating with a few drops of H_2O_2 until a clear solution resulted. These remains and all other liquids resulting from the iodine separation were preserved to be analyzed for other radioisotopes.

The I^{131} on SAE_1 was obtained as follows: (1) Set up triple column (SCE_1, SAE_1, and WAE_1) that had been loaded at sea. (2) Add 1 ml of each carrier solution (10 mg/ml; I^-, Co^{++}, Fe^{+++}, Sr^{++}, Ba^{++}, and Zn^{++}) and draw into column. Load cations onto SCE_1 and I^- onto SAE_1 by passing 50 ml 0.5 $\underline{\underline{N}}$ HCl through triple column. (3) Separate resins; store SCE_1 and WAE_1 for later analyses (see D, page 16). Transfer SAE_1 to a new column. (4) Elute I^- from SAE_1 with 60 ml 9 $\underline{\underline{N}}$ HCl; store resin for later analyses. (5) Neutralize the eluate with NaOH and add 1 ml of 6 $\underline{\underline{N}}$ HNO_3 and 1 ml 1 $\underline{\underline{M}}$ $NaHSO_3$ to assure complete reduction to iodide; (6) Transfer solution to separatory funnel and add 10 ml of CCl; add 1 $\underline{\underline{N}}$ $NaNO_2$ slowly

until I_2 appears in solution and extract into CCl_4. (7) Withdraw CCl_4 and perform 3 to 5 additional extractions during cycles of reduction to I^- with $NaHSO_3$ followed by oxidation to I_2 by $NaNO_2$ until no more iodine is recovered. Combine CCl_4 extracts for the final steps of purification below.

The I^{131} in the $Effluent_1$ was obtained by solvent extraction. (1) Transfer $Effluent_1$ and bottle rinsings to a liter flask. (2) Add 26 ml 2 $\underline{M}$ Na_2CO_3, 4 ml 0.09 $\underline{N}$ KI carrier and 2 ml NaOCl solution (5-6% available Cl_2) to oxidize all iodine to IO_3^-. (3) After an hour or longer, transfer solution to separatory funnel. (4) Add 8 ml 16 $\underline{N}$ HNO_3 and 1 $\underline{M}$ $NH_2OH \cdot HCl$ until I_2 color persists (> 2 ml); extract I_2 3-4 times into 10 ml CCl_4. These extracts were combined for the final purification described below. The aqueous phase of $Effluent_1$ was returned to its polyethylene bottle for later analyses for other radioisotopes.

The final steps in purification and the counting of I^{131} from the tissues, the SAE_1 and the $Effluent_1$ were done as follows: (1) Transfer solution of I_2 in CCl_4 to separatory funnel. Add 15 ml distilled water. Add 6 drops 1 $\underline{M}$ $NaHSO_3$ and extract I^- into H_2O. (2) Add 1 ml 6 $\underline{N}$ HNO_3 and 6 drops 1 $\underline{N}$ $NaNO_2$ to aqueous phase. Extract I_2 into 10 ml CCl_4. (3) Repeat step (1). (4) Add 1 ml 6 $\underline{N}$ HNO_3 to aqueous phase and make up to 25 ml with distilled water. (5) Add 2 glass boiling-beads, bring to boil, and add 3 ml 0.1 $\underline{N}$ $AgNO_3$ dropwise with stirring. Boil several minutes to digest AgI precipitate. (6) Filter on tared Whatman No. 1 paper, remove beads, rinse precipitate with 95% ethyl alcohol, dry, and weigh. (7) Transfer AgI and filter paper to counting tube and count I^{131} in well-type scintillation detector.

Separation of Barium-140. Immediately following the analyses for I^{131} we looked for this 12.8 day isotope on six of the SCE_1. The SCE_1 was eluted

with 40 ml 9 N HCl, this HCl then being passed through the SAE_1 to recover Mn, Fe, Co, and Zn. (These radioactive metals from SCE_1 and SAE_1 were combined by this procedure for these six experiments. This was unfortunate because they were among the most radioactive samples and therefore could have shown anionic to cationic form of excretory products better than some other samples.) The 9 N HCl effluent was bottled and set aside. The barium was then eluted from the SCE_1 with 100 ml 0.5 N ammonium citrate. The eluate was dried with HNO_3, then ashed in a muffle furnace at 700°C. The ash was dissolved in 4 ml 9 N HCl, $BaCl_2$ was precipitated with cold HCl-ether, then redissolved in distilled water. The La^{140} was scavenged with $Fe(OH)_3$ precipitate, and then H_2SO_4 was added to the supernatent to precipitate $BaSO_4$. This was filtered, dried, weighed, and counted. The recovery was satisfactory but the activity of the samples was so low that no further analyses were done. No data will be presented.

Separation of Sr, Mn, Co, Fe, and Zn. The separation of these elements was based on the varying affinities of their chloride complexes for strong anion exchange resin (Kraus and Nelson 1956). The preliminary steps were the same for all 5 elements but different for each type of sample; the final steps were different for each element but the same for all types of samples.

A. Tissues. Tissue samples fell into two groups.

I. The procedure for tissues previously analyzed for I^{131} (therefore wet-ashed in H_2SO_4)was as follows: (1) Place samples in beaker with 15 ml 9 N HCl. Add 1 ml Mn, Co, Fe, Zn, and Sr carriers (10 mg/ml) and dry under lamps. (2) Dissolve dry salts in HCl and make up to final concentration of 0.5 N HCl. (3) Load cations (0.5 ml/min) onto SCE_2 column previously equilibrated with 0.5 N HCl. The subsequent steps for each element are discussed below.

II. Animals still in formaldehyde were identified and enumerated, weighed damp-dried and oven-dried (90°C), and each species placed in a small porcelain crucible. If all specimens from an experiment were the same species, the formaldehyde was added; if more than one species had been used, the formaldehyde was analyzed separately. One-ml portions of Mn, Co, Fe, Zn, and Sr carriers were added. The samples were dried, ashed (700°C), dissolved in 5 $\underline{N}$ HCl on a hot plate or steam bath, dried again to chloride salts, redissolved in 0.5 $\underline{N}$ HCl, and loaded onto a SCE_2 column as above. Subsequent steps are given below.

B. Membrane Filters. The particulate excreta was analyzed as follows: (1) Cut filter into bottom of porcelain crucible and burn it away with a small gas flame. (2) Add 1 ml of Mn, Co, Fe, Zn, and Sr carriers and dry. Complete the ashing in muffle furnace at 550°C. (3) Dissolve ash in HCl and evaporate to dryness. (4) Redissolve salts in 0.5 $\underline{N}$ HCl and load onto SCE_2 column as above. Subsequent steps are given below.

C. $Effluent_1$. The $Effluent_1$ samples, whether previously analyzed for I^{131} or not, were transferred to beakers, dried under lamps and handled as follows: (1) Transfer the salts to crucibles and add 1 ml of Mn, Co, Fe, Zn, and Sr carriers. (2) Dry, then ash salts at 550°-600°C (higher temperature caused salts to fuse). (3) Transfer back to beakers, dissolve in 10 ml 12 $\underline{N}$ HCl plus several drops 30% H_2O_2 to form oxidized metal chlorides. (4) Dissolve each sample in distilled water or dilute HCl until final solution is 0.5 $\underline{N}$ in chloride, including the Na and Mg chlorides from the original 400 ml of sea water. (5) Load the filtrate onto a SCE_2 column as above. Subsequent steps are given below.

D. SCE_1, SAE_1, and WAE_1 Resins. The following is the procedure used for most of the original ion exchange resins: (1) Transfer resin into crucible and dry under lamp. Char resin in muffle furnace slowly raised to 550°C. (2) Dissolve ash in 9 $\underline{\underline{N}}$ HCl and dry down again. Transfer dry salts to beaker and add 1 ml of Mn, Co, Fe, and Zn (also Sr to some SCE_1 samples) carriers. Add 10 ml 12 $\underline{\underline{N}}$ HCl and dry again. (3) Dissolve in 0.5 $\underline{\underline{N}}$ HCl and load onto equilibrated SCE_2 column. Subsequent steps for each element are given below.

The Mn, Co, Fe, and Zn that had been in tissues, on membrane filters, on SCE_1, SAE_1, or WAE_1, or in the $Effluent_1$ were now separated as follows: (1) Mount the newly loaded SCE_2 columns directly over a clean SAE_2 column equilibrated with 9 $\underline{\underline{N}}$ HCl. (2) Pass 40 ml 9 $\underline{\underline{N}}$ HCl through this double column, eluting Mn, Co, Fe, and Zn from the SCE_2 and loading them on the SAE_2 as chloride complexes. (Part of the strontium was eluted by 9 $\underline{\underline{N}}$ HCl but it was not held by SAE_2; see below). Remove the SCE_2 resin and the 9 $\underline{\underline{N}}$ HCl effluent and store separately for strontium analyses. (3) Selectively elute the four metals from the SAE_2 with decreasing concentrations of HCl in four steps: Mn^{++} in 7 $\underline{\underline{N}}$, Co^{++} in 4 N, Fe^{+++} in 0.1 $\underline{\underline{N}}$, and Zn^{++} in 0.005 $\underline{\underline{N}}$ HCl. At least 30 ml of each HCl solution was used, but as much as 50 ml was used if the column or effluent showed that Co (pink) or Fe (yellow) was still being eluted after the first volume. The final elution for Zn was repeated occasionally if recovery of carrier indicated substantial loss. (4) Purify, precipitate, weigh and count each element by procedures given below.

There were several deviations from this general procedure for ion exchange resins. (1) The loading of carriers onto SCE_1 and SAE_1 for the I^{131} samples was described above. (2) The handling of SCE_1 and SAE_1 for

the Ba^{140} samples has been described. (3) In the case of four samples both the SCE_1 and the 9 N HCl effluent that has been used to carry the Mn, Co, Fe, and Zn onto SAE_2 were combined in a search for radioactive strontium (see below). Although there were variations from the standard procedure as we investigated potentially interesting isotopes, the early addition of a known amount of carrier and a final correction for the amount of carrier recovered give assurance that no uncorrected losses occurred.

Purification of Strontium-89,90, and Yttrium-90. The SCE_2 resins that had been eluted with 9 N HCl were muffled and the ash dissolved in HCl and dried. To this was added the 9 N HCl effluent from the SAE_2 resin (see p. 16) and the sample was redried. The purification technique for Sr followed Procedure 20 of Sunderman and Townley (1960), except that there was a prior precipitation of $SrCO_3$ and an intermediate alcohol-ether extraction of calcium. The final $SrCO_3$ was secured to the filter paper after weighing by drawing through dilute collodion, and counted with a gas flow detector.

The $SrCO_3$ was dissolved off the filter paper and the Y^{90} that had grown in during a 2-week period was scavenged out by $Fe(OH)_3$ precipitation. This precipitate was mounted and counted by an end-window beta detector surrounded by anti-coincidence chambers (designed and built by Dr. Victor Noshkin, WHOI) and having a background of approximately 0.3 cpm.

The counts of $Sr^{89,90}$ and Y^{90} were not detectably above background and the data will not be presented.

Purification of Manganese-54. After elution from SAE_2 resin with 7 N HCl, the Mn was carried through the following procedure to assure removal of possible contaminants such as Co, Zr, and Nb. (1) Dry 7 N HCl effluent under lamps. Dissolve salts in 20 ml 2 N HNO_3 and add 1 ml $CoCl_2$ carrier (10 mg/ml). (2) Add 0.3 g dry $NaBrO_3$ and heat in water bath 30 minutes to precipitate MnO_2. Centrifuge MnO_2 and wash three times with distilled

water. (3) Dissolve MnO_2 in 10 ml 2 $\underline{N}$ HCl plus 1 drop 30% H_2O_2. Remove excess H_2O_2 by boiling sample. Cool and transfer to separatory funnel. (4) Add 0.5 mg $FeCl_3$ carrier and 6 mg cupferron. Extract Fe (also possible Zr and Nb) into 2 ml chloroform repeatedly until green color of chloroform shows all iron is extracted. (5) Dry aqueous layer under lamps. Dissolve salts in 20 ml 2 $\underline{N}$ HNO_3 and add dry $NaBrO_3$ to precipitate MnO_2. Centrifuge MnO_2 and wash twice with distilled water. (6) Dissolve MnO_2 in 2 drops 9 $\underline{N}$ HCl plus 1 drop 30% H_2O_2. Dry, then dissolve in distilled water. (7) Add 5 ml 2 $\underline{N}$ $(NH_4)_2HPO_4$. Add NH_4OH dropwise until NH_4MnPO_4 precipitates. Filter through Millipore® filter. (8) Char filter and precipitate in crucible, and ignite over Fisher burner at 1000°C for 1 hour to convert to $Mn_2P_2O_7$. (9) Weigh $Mn_2P_2O_7$ to determine recovery. Count in scintillation well detector at 0.84 Mev peak.

Purification of Cobalt-58,60. Possible contaminants were removed and cobalt isotopes prepared for counting as follows: (1) Dry 4 $\underline{N}$ HCl eluent from SAE_2 resin under lamp. Dissolve salts in 10 ml 2 $\underline{N}$ HNO_3. Add 1 drop Mn carrier and dry $NaBrO_3$ to precipitate any possible Mn contamination. Filter off MnO_2. (2) Add 1 drop Fe carrier to filtrate and precipitate it with NH_4OH. Filter off this $Fe(OH)_3$ scavenging precipitate. (3) Add 3 ml 3.5 $\underline{N}$ $(NH_4)_2HPO_4$ to the filtrate. Filter off NH_4CoPO_4. (4) Ignite over Fisher burner at 900°C for 1 hour to convert NH_4CoPO_4 to $Co_2P_2O_7$. Weigh sample, then count all gamma radiations from 0.76 to 2.00 Mev to include peaks from Co^{56}, Co^{58}, and Co^{60}. Co^{56} was not distinguishable from Co^{58} in our samples and probably was much less abundant; it will therefore not be separately discussed. Cobalt radioactive decay corrections were based on the Co^{58}/Co^{60} ratios of other plankton labeled by nuclear testing (Lowman, 1958). From his ratios we calculated that,

initially, Co^{58} would be about 94% of Co^{58} + Co^{60} count rate. Our samples were counted almost a year after our reference date (13 June 1963) and our correction factor for decay of both Co^{58} and Co^{60} was 10.

Purification of Iron-55,59. The iron isotopes were prepared for counting as follows: (1) Dry the 0.1 N HCl eluent from SAE_2 under lamp. Dissolve salts in 15 ml 6 N HCl. (2) Transfer sample to separatory funnel and extract 3 times with 5 ml ethyl ether. Wash combined ether fractions with 6 N HCl. (3) Extract iron from ether with three 5-ml portions of distilled water. (4) Precipitate $Fe(OH)_3$ with NH_4OH. Centrifuge. Wash precipitate with 1 N NH_4OH and filter onto Millipore® filter. (5) Place filter in small crucible and ignite carefully with a small flame. Place crucible in muffle furnace at 700°C for 30 minutes to convert $Fe(OH)_3$ to Fe_2O_3. Weigh Fe_2O_3. (6) Count 1.29 Mev gamma peak for Fe^{59}. (7) Transfer Fe_2O_3 to center area of planchet and secure in position with disc of Mylar® film (1.0 mg/cm^2) cemented around edge of planchet with collodion. Count Fe^{55} in gas flow detector and correct for mylar absorption (26%), self absorption (proportional to sample weight; factors obtained experimentally), and detector efficiency.

Purification of Zinc 65. The zinc radioisotope was purified for counting by procedures of Freiser (1962), Hicks (1960), and Lundell and Hoffman (1938). (1) Dry the 0.005 N HCl eluent from the SAE_2. Dissolve salts in 2 N HCl. (2) Transfer to separatory funnel. Add 120 mg cupferron and 2 ml $CHCl_3$; extract iron and other cupferrate complexes three times. Wash with 2 ml $CHCl_3$ three times, or until $CHCl_3$ is green. Dry the aqueous phase. (3) Dissolve salts in 25 ml 0.1 N HCl. Heat to 60°C and add 1 ml 8-quinolinol (5% in 2 N acetic acid) and 25 ml 2 N ammonium acetate. Digest at 60°-70°C; cool. (4) Filter onto washed-and-weighed glass-fiber filter. Dry and

weigh. (5) Count Zn^{65} at 1.12 Mev peak.

In addition to the experiments performed aboard PIONEER, a number of measurements of I^{131} and CO^{58} elimination by zooplankton were performed aboard ANTON BRUUN in the Indian Ocean in early 1964. These experiments differed from the previous series in several respects. First, the animals were unlabeled when captured; therefore, after sorting into species, they were placed in a solution of one of the isotopes in sea water until they had taken up at least 500 counts per minute per experiment. The animals were then placed into about 80 ml of clean sea water. At the end of the excretion period this water was passed serially through a membrane filter, then 6 ml of SCE, 5 ml of SAE, and 5 ml of WAE at 0.8 ml/min. Usually the same labeled animals were used for two consecutive excretion experiments to find if the excretion rate changed with time after labeling. Finally, the radioactivity of the animals, the filters, the three ion exchange resins, and the effluent was measured aboard ship using a scintillation crystal and scaler as described above for gross gamma (p. 10). Background at sea was relatively constant at about 140 cpm.

IV. Efficiency of Ion Exchange Resins.

The concentrations and complexities of the ions in sea water make it difficult to predict the major chemical species present (see discussion below). Therefore it was not known whether our isotopes would be quantitatively removed by passage through an ion exchange column. Our approach to this problem was empirical. A small amount of I^{131}, Co^{58}, Mn^{54}, Fe^{59}, or Zn^{65} was added to 200 ml of filtered (0.45μ Millipore®) sea water and the solution was passed through 5 ml of SCE or SAE resin at 0.5 ml per minute. Fractions were collected and counted to determine the point and extent of breakthrough. From these experiments we learned the retention

of inorganic ions on resins and thus have reference against which to compare the behavior of the isotopes excreted by zooplankton.

RESULTS

I. Diurnal Plankton Migrations.

The plankton obtained in the quantitative hauls was very diverse. There were many species from many phyla differing in size, morphology, and ecological niche. Number of individuals and wet weight per 100 m^3 are used here to describe population densities. It is recognized that these are not completely comparable from one group of animals to another. The species enumerated in Figures 2-6 described below are the dominant ones only, whereas the weights given are for all species. These dominant species probably account for more than 90% of the total weight, except for the Chaetognatha, where only about 70% of the animals were identified and enumerated. The diurnal changes in population density in the upper 100 m was considered most critical because this was the approximate thickness of the mixed layer above the thermocline. Thus, contaminants introduced at the surface would not mix rapidly by eddy diffusion to greater depths; biological transfer, however, might prove significant.

Copepoda ranked first in wet weight in the upper 100 m where they were most abundant (Table 1). Some evidence of diurnal migration is gained from wet weights of total copepods; counts of individual species showed definite migration in the important genus Pleuromamma and probably in Euchaeta media, confirming reports of Esterly (1912), Moore (1949) and others. Pleuromamma will be discussed below.

The colonial tunicate Pyrosoma verticillatum ranked second in wet weight; it was found in the upper 100 m (Table 2) throughout the day. Siphonophora

were the third heaviest group. Moore (1953) found evidence of diurnal migration in siphonophores, and Barham (1963) suggested that diurnal changes in depth of the deep scattering layer might be the result of their migrations but gave no supporting quantitative data. The large changes in mass at the surface in Table 3, however, indicate that migration occurs in some siphonophores. The next largest group in terms of wet weight was Salpidae-Doliolidae. Thalia democratica showed a little evidence of moving down to 300-500 m at midday; total weights also increased with depth at midday (Figure 2). The three groups, Pyrosomatidae, Siphonophora, and Salpidae-Doliolidae, are quite watery and they appear more important judged by wet weight than they would judged by dry weight, protein content, or another index of metabolic activity. Furthermore, the volume of water sampled by our nets was small and did not adequately integrate their distribution; an occasional large individual would distort the weight distribution from that expected from the number of individuals.

Most of the Chaetognaths stayed in the upper 100 m (Figure 3). The weights and counts agree in depicting vertical migration in the Euphausiidae (Figure 4), the sixth ranked group; Moore (1949) and Brinton (1962a, 1962b) also reported diurnal migration. The medusae were the next most abundant and there is some indication of vertical migration (Table 4). Here again, however, our net did not adequately sample them and an occasional large individual could easily have distorted the weight distribution. Changes in the weight distribution of ostracods plus other small crustaceans throughout the day (Table 5) suggests that about two-thirds of them move out of the upper 100 m at mid-day. The copepod genus Pleuromamma showed definite migration (Figure 5). Weights of Pleuromamma were not obtained on the samples; from wet weights of adults times their numbers, however, the

surface layer contained 74 mg/100 m^3 between 2100 and 0300 but only about 2 mg/100 m^3 during the day. Migration by members of this genus alone, however, could not account for the differences in biomass in the upper 100 m seen in Table 1. Ranking tenth in wet weight were the Pteropoda; they also migrate significantly (Figure 6). Finally, the mass of fishes caught in the surface water at night was much greater than at mid-day (Table 6).

II. Levels of Radioactivity in Zooplankton.

It is well known that radioactivity released into natural environments is usually greatly concentrated in the biota. Table 7 shows the gross gamma activity per gram wet weight of animals used for excretion experiments during the eleven day period aboard PIONEER. All counting was done June 13-15, 1962. The day to day changes in levels of radioactivity within any taxonomic group undoubtedly were the result of sampling several populations, each of which was exposed to different concentrations of radioactivity for varying periods of time. Comparison of one group to others collected at the same time shows that pteropods accumulate more activity per unit body weight than any other kind of organism. Crustaceans --- euphausiids, amphipods, decapods, Candacia, and mixed plankton (largely copepods) --- also generally had high count rates. These results confirm the findings of Berner, et al. (1962) that ciliary and mucous feeders, such as pteropods, accumulate large amounts of radioactivity quickly. It should be noted that the amount of activity per unit of organic matter would be much higher in "watery" forms such as ctenophores, pyrasomas, scyphazoans, and salps. The zooplankton taken below the thermocline at dawn on days 4 and 5 (Table 7) contained high levels of activity; this proves qualitatively that diurnal migration of zooplankton rapidly transports radio-activity through the thermocline.

The activity attributable to each of the isotopes separately counted shows the same pattern as the gross gamma activity (Table 8). Except for the copepods (Candacia ethiopica collected at 300 m on day 4), on any given day the pteropods had more of every radioisotope per unit body weight than did any other group of animals. Crustaceans generally accumulated relatively large amounts of activity also. In general the amount of each isotope declined with time; the animals were exposed to successively lower levels of activity in the water because of dispersion and dilution of the original pool. The five elements studied individually accounted for only a small portion of the gross gamma activity (Table 7).

III. Elimination Rates.

It seems evident that not all the radioactivity lost from experimental animals represents true excretion. Some of the particulate matter and some of the soluble fractions recovered probably never formed part of any metabolic pool within the animal. For this reason, the terms elimination or loss are preferable to excretion until we learn the pathways by which these elements leave the organism. A separate project now in progress at Woods Hole Oceanographic Institution is concerned with the route by which certain radio-isotopes enter zooplankton and the sites in the animals where they are most concentrated. Data from this study will help determine how much represents true excretion in some of the animals.

The conversion of the radioactivity of various eliminated products to the elimination coefficient, λ, permits averaging of experimental results from the same kinds of animals. It also permits the comparison of rates involving one kind of animal or isotope with those of others. The unit of λ is hr^{-1}; a λ of 0.01 means that the animal is losing 1% of a particular isotope per hour.

Iodine. When sea water containing I^- or IO_3^- (carrier-free I^{131}) was passed through SCE resin there was no uptake of iodine (Figure 7). However, when passed through SAE resin, I^- was quantitatively removed from sea water whereas IO_3^- broke through immediately (Figure 7).

The short half-life of I^{131} permitted gaining only fragmentary data on elimination rates from the PIONEER samples; data from experiments on copepods aboard ANTON BRUUN are also shown (Table 9). With the exception of Pyrosoma, the largest pool of excreted iodine was taken up by SAE_1, and was, therefore, probably iodide. The inability to detect I^{131} on SAE, in the Pyrosoma experiments is possibly a consequence of having performed these iodine separations last, after most of the isotope had decayed. From the small amounts of I^{131} appearing on the MF or WAE_1 in the ANTON BRUUN experiments, failure to examine these fractions in the PIONEER experiment may not have caused much error. On the other hand the ANTON BRUUN copepods eliminated a significant amount of I^{131} in a form with an affinity for SCE_1, but no I^{131} appeared in the $Effluent_1$. Some indication of the time-course of loss of a single dose of iodine is obtained from the ANTON BRUUN experiments; the rate during the first 5-8 hour period is 5-6 times higher than during the second period. Finally, it may be seen that total elimination rates during experiments lasting 0.2-1.0 day were about 1-6% per hour. The total rates might have been higher in the third group of pteropods, the ctenophores, and the mixed plankton (mostly copepods, but a few chaetognatha and small euphausiids) if we had analyzed SAE_1 before the I^{131} decayed away.

Manganese. When sea water containing carrier-free Mn^{54} was passed through 5 ml of SCE resin, radioactivity began to appear after 25 ml and reached the activity of the incoming solution after 70 ml (Figure 8); breakthrough occurred almost immediately with SAE but the activity of the effluent never

reached that of the incoming solution. From the curves, about 15% of inorganic manganese may be retained by SCE and about 25% by SAE when 400 ml of sea water is passed through 15 ml of the resins.

The Mn^{54} levels were low in all of the PIONEER samples and only limited conclusions can be drawn concerning elimination (Table 10). Most of the eliminated manganese was caught on the membrane filter. Therefore it was either already in particulate form, perhaps in the feces, or was precipitated soon after excretion. Unfortunately we cannot determine whether the SCE_1 or the SAE_1 exchanged the major fraction of soluble Mn^{54} from Pyrosoma since these resins were combined. The total elimination rate of pteropods and pyrosomas was in the range of 1-5% per hour.

Cobalt. When sea water containing Co^{58} was passed through 5 ml of SCE resin, breakthrough began almost immediately and the effluent activity reached about 90% of the incoming activity after 100 ml had passed through (Figure 8). Breakthrough began immediately with SAE resin, but after reaching 70% the effluent activity went down to about 50% of the incoming activity (Figure 8). The changing form of the SAE resin as the sea water passed through, perhaps by adsorption of organic complexing substances, seemed to improve its affinity for cobalt. From the curves we would expect about 33% of inorganic cobalt to be retained by SCE resin and 55% of the remainder by SAE resin when 400 ml of sea water is passed consecutively through 15 ml of these resins (PIONEER). On the other hand, about 70% would be retained on either SCE or SAE when 80 ml of sea water was passed through 6 ml of resin (ANTON BRUUN).

Data on cobalt elimination were obtained from PIONEER and from ANTON BRUUN (Table 11). In most experiments a significant amount of cobalt appeared in particulate form; this was the major fraction from the PIONEER

pteropods. Furthermore the particulate activity might have been even higher; storage of the filters in formaldehyde may have permitted some cobalt to be dissolved. The WAE_1 took up little activity in any experiments, the soluble cobalt generally appearing in about equal amounts in $Effluent_1$ and on SCE_1. In some of the ANTON BRUUN experiments samples of the effluent were shaken with additional SCE, and this fresh resin usually took up most of the effluent activity. From this, and breakthrough experiments reported above, we conclude that the effluent activity was probably Co^{++} displaced from the SCE by the high concentrations of Na^{+} and Mg^{++}. As with iodine, copepods lost cobalt much faster during the first of the consecutive experiments. Pyrosoma had a lag phase and the excretion rate was higher during the second 10-hour period. Total elimination rates for all the animals were usually 1-6% per hour.

Iron. When Fe^{59} was put through a SCE resin, breakthrough started early, but the effluent never reached more than 20% of the level of incoming solution. (Figure 9). This may only indicate that the flow rate was a little too rapid. Iron was almost completely removed by SAE resin, however. From these curves it may be expected that 85% of all inorganic iron would be removed from SCE and 95% of the remainder by SAE when 400 ml of sea water passed consecutively through 15 ml of these resins.

The Fe^{59} levels in the PIONEER samples were undetectable except in the tissues and some MF samples. In general they agree with the Fe^{55} results, however, and will not be presented. The Fe^{55} was eliminated both in particulate and soluble forms (Table 12). It is unknown whether the Fe^{55} on MF was particulate when eliminated or if it precipitated from some soluble form during the experiment. The particulate Fe^{55} also might have been higher before the filters were stored in formaldehyde. From the Salpa

and the second Pyrosoma experiments it appears that the exchangeable iron was strongly anionic; perhaps the exchangeable iron from pteropods and the other pyrosomas were also anionic. The nature of anionic iron in sea water is unknown, but organic complexes have been suggested (Lewis and Goldberg 1954) and are certainly possible in animal excreta. The non-exchangeable iron in the effluent may be particles less than the pore size of MF (0.8μ) or uncharged complexes. Total elimination rates were 1-8% per hour.

Zinc. Zn^{65} in sea water breaks through SCE resin after 35 ml and reaches the level of the incoming solution after 145 ml; Zn^{65} is quantitatively removed from SAE resin (Figure 9). From the SCE curve it appears that about 70% of any inorganic zinc would be removed from 400 ml of sea water by 15 ml of this resin.

Zinc activity was high compared to other isotopes in PIONEER zooplankton and elimination measurements were possible on a variety of animals (Table 13). Here again, appreciable amounts were particulate and only small amounts were exchanged on WAE_1. Storage of the filters in formaldehyde might have dissolved away some of the particulate zinc. Significant amounts were exchanged on the other two resins, SAE_1 apparently being more retentive. Since inorganic zinc in sea water exchanged on both SCE_1 or SAE_1 resins, this is evidence that zinc is eliminated from zooplankton predominantly as inorganic ions. The measurable amounts of zinc that went through the SAE_1 and were found on WAE_1 and in $Effluent_1$ indicate that some soluble non-exchangeable products are also eliminated. The zooplankton zinc pool appears to be slightly less labile than others, with total elimination rates generally amounting to 1-4% per hour.

DISCUSSION

I. Elemental Turnover Rates in Zooplankton.

The best comparisons of different elements in each zooplankton group can be made using the data from ANTON BRUUN and the first four experimental days aboard PIONEER (Table 14). Pteropoda had elimination rates of 3-6%/hr for Mn, I, and Fe, 2-4%/hr for Co, and about 1%/hr for Zn. Pyrosoma had rates of 3-5%/hr for Mn and Fe, 2-3%/hr for Co, and 1%/hr , or less, for I and Zn. The rates of elimination of Zn from euphausiids, 3-8%/hr, are very high compared to those of pteropods and pyrosomas for this element; this evidence for high rates conflicts with the indirect evidence of Osterberg, Pattulo, and Pearcy (1964) that the biological half-life of Zn^{65} in Euphausia pacifica is relatively long. Finally, in the three species of copepods, elimination of I occurred at rates of 1-6%/hr and Co at 4-6%/hr. The almost 3-fold higher rates of I elimination by Pleuromamma xiphias than by P. abdominalis indicates a need for more intensive study of physiological differences and also warns of the danger in extrapolation of results from one species to another, even within the same genus.

In addition to differences directly related to the physiology of mineral balance in zooplankton there are two factors related to the timing of the experiments. There was a generally lower rate of elimination of radio-isotopes in the later PIONEER experiments than in early ones, with the possible exception of pyrosomas and chaetognaths (Table 14). This is to be expected if one assumes that isotopes become incorporated into less labile pools within the animals following repeated entrance into, and feeding in, the contaminated layer. Assuming this phenomenon occurs generally, then we would expect that the elimination rates of Zn in

chaetognaths and salps, and of Fe in salps, were very high during the first few days. Secondly, the data obtained from consecutive excretion experiments with the same labelled individuals aboard ANTON BRUUN (Tables 9 and 11) showed that elimination rates are not constant following a single dose of I^{131} or Co^{58}. In copepods, the loss rates of iodine and cobalt declined with time. This has also been shown for Zn^{65} in fish (Chipman, Rice, and Price, 1958) and snails (Mishima and Odum, 1963). If this phenomenon is general, the initial rates will be underestimated in long-term experiments. High turnover rates of elements in the dominant species of the plankton probably are more important ecologically than they are physiologically and should be studied from this standpoint.

Rates of 1 to 6% per hour represent turnover times of 100 to 17 hours, respectively, or between 4 and 0.7 days. Turnover times of this order have been reported by, or can be calculated from the data of, several workers who have studied small animals (Table 15). The "fast reaction", or "labile pool", reported by Conover (1961) and others was also found in the ANTON BRUUN experiments and it is evident that loss of an isotope during the interval immediately following labeling is much more rapid than it is several days later. The graphical separation of a curve that might represent a continuum of processes into simply two or three processes was criticized by Van Liew (1962), however. There are almost certainly many pools for each element within an organism, each being cycled in some time interval, dependent on both the pool size and the flux rates affecting it. Our data do not give details on these pools, but they do show that significant amounts of elements circulate rapidly through zooplankton. Actually, most animals probably have numerous very small pools that turn over so rapidly that they have not yet been satisfactorily measured. The washing of labeled animals prior to

beginning an experiment may virtually exhaust pools with high flux rates. Small pools with high flux rates (for example, materials sorbed to the surface of an animal, or to bacteria on the surface) may be of little importance in the physiology of any one animal, but they may significantly influence the state or location of the element in sea water.

II. Physical and Chemical Form of Eliminated Elements.

The form in which an element is eliminated by an animal may depend on several factors. It may be influenced first by the form in which the animal obtains the element. For example, the animal might ingest an inorganic particle of low digestibility or an easily digestible plant cell. It might swallow water containing dissolved ions or exchange such ions across gill or other membranes exposed to the water. It might simply become contaminated by adsorption or exchange in the mucous coating of the body. It may be influenced by the type of site where the element is concentrated, or by its particular physiological function - enzyme activator, metalloporphyrin, or vitamin constituent. The literature provides no data to be compared with our results on the state of these elements when eliminated by zooplankton. It is possible, however, to compare our results with the data and calculations of Sillén (1961) on the elemental states normally present in sea water.

There are several factors that influence the ion exchange behavior of elements when dissolved in sea water --- relative adsorption, ionic form, and concentration of competing or complexing ions. The relative adsorptions of Mn^{++}, Co^{++}, Fe^{+++}, and Zn^{++} are all much higher than that of Na^{+}, but the Na^{+} concentration is high enough to compete effectively (Table 16). The relative adsorption and concentration of Mg^{++} are also sufficient to displace all the above metals, except perhaps Fe^{+++}. The

breakthrough experiments shown in Figures 7, 8, and 9 corroborate these expectations.

These experiments also tend to confirm some of the physical-chemical predictions of Sillén (1961) on the probable form of these elements in sea water. Manganese dissolved in sea water broke through both SCE and SAE resins relatively soon (Figure 8) and thus might exist, at least in part, as some uncharged hydroxide species. Most of the manganese eliminated by zooplankton was particulate (Table 10); the soluble part was insufficient to show if it differed from ordinary inorganic dissolved manganese. Inorganic cobalt was retained much better by SCE resin than by SAE resin (Figure 8). This was also true of the cobalt eliminated by zooplankton (Table 11). The amount of radiocobalt found in $Effluent_1$ (Table 11) can simply be attributed to inorganic cobalt breaking through both resins. The predominant form of iron in sea water was not clear to Sillén; he suggested uncharged $Fe(OH)_3$. The strong adsorption by both SCE and SAE resins (Figure 9) indicate that it is either amphoteric or else it exists in two or more forms or complexes in sea water. The results in Table 12 do not support the hypothesis that iron is largely eliminated in a simple inorganic form. It is possible that much of the Fe^{55} found on MF was adsorbed ionic or colloidal rather than entirely particulate. The failure of SCE_1, to remove the Fe^{55} before it went onto the SAE_1 or into $Effluent_1$, however, suggests that some of the eliminated iron was in an anionic or neutral organic complex.

The relatively slow breakthrough of zinc on SCE and complete retention on SAE (Figure 9) is not satisfactorily explained by Sillén's prediction of the ^{+}Zn form. Sillén and Liljequist (1944) calculated the formation constants of the complexes $ZnCl^{+}$, $ZnCl_2$, and $ZnCl_3^{-}$, and recognized that $ZnCl_4^{=}$ might also

be important at the chloride concentrations they employed (< 1M). Assuming rapid formation of these anionic chloride complexes, quantitative removal of zinc by SAE might take place even though the complexes were not the dominant form of zinc in solution. The finding of eliminated Zn^{65} on both SCE_1 and SAE_1 resins (Table 13) suggests elimination of inorganic Zn^{++}. The finding of significant amounts of Zn^{65} on WAE_1 and in $Effluent_1$, however, after passage through SAE_1 suggests that zinc also may be organically complexed when eliminated by zooplankton.

The physical-chemical calculations of Sillén (1961) showed that IO_3^- would be much more important in sea water than I^-, and he suggested that earlier experimental evidence for abundant I^- resulted from nonequilibrium and perhaps some experimental difficulties. For example, Barkley and Thompson (1960) reported that only one- to two-thirds of the iodine of North Pacific Ocean water was IO_3^-. In the Arctic Ocean, however, IO_3^- increased with depth, from zero at the surface to 100% of the total iodine at 500-1000 m. In both oceans total iodine remained fairly constant except in regions of high biological activity. The results in Table 9 and Figure 7 indicate that most iodine was eliminated as I^- and a smaller amount (undetectable in the copepods from ANTON BRUUN) was eliminated as, or was converted to, IO_3^- or another non-exchangeable form. The cationic I^{131} from the ANTON BRUUN copepods presumably was organically complexed. Gorbman, Clements, and O'Brien (1954) concluded that much of the iodine taken up by aquatic invertebrates was protein-bound, especially in exoskeleton and epithelium. Monoiodotyrosine, diiodotyrosine, thyroxine, and diiodothryonine were found in molluscs and annelids; our cationic I^{131} might have been such substances.

III. Elemental Turnover Rates in Sea Water.

Cobalt is the element for which most data are available to calculate the effect of zooplankton on elemental cycling in the upper layers of the sea, and transport through the thermocline. The cobalt content of the important zooplankton groups (Table 17) were obtained as follows. Nicholls, Curl, and Bowen (1959) reported analyses on these species: Calanus finmarchicus (group (1) Table 13), Salpa fusiformis (4), Sagitta elegans (5), Euphausia krohnii (6), Cyanea capillata (7), Centropages typicus and C. hamatus (9), and Limacina retroversa (10). Parker, Gibbs, and Lawler (1963) reported cobalt content for Physalia, group (3). Cobalt content of fish recently was reported to be from 0.01 ppm (Tsuchiya and Makuta 1951; mean of 26 spp.) to more than 1 ppm (Parker, et al. 1963; 3 spp,); an intermediate value, 0.1 ppm fresh weight, was chosen for group 11. Finally, Pyrosoma, group (2), was considered to have about the same amount of cobalt as Salpa. For lack of any data, the value for Ostracoda and other crustaceans, group (8) was set between that of Copepoda and Euphausiidae. The total elimination rates, $\Sigma\lambda$, not in parentheses (Table 17) are weighted means from Table 11, using only the initial rates (I_0-T_1) in the ANTON BRUUN experiments, and using the $\Sigma\lambda$ for Neocalanus gracilis as the rate for Copepoda. The zooplankton groups for which experimental data are not available have been assigned a $\Sigma\lambda$ of 0.03 hr^{-1} (Table 17, in parantheses).

Zooplankton weights (from Tables 1-6 and Figures 2-6 are wet weights per square meter in the upper 100 meters depth, or weight/100 m^3. Pleuromamma weights have been subtracted from total copepod weights (Table 1). The minima and maxima mostly occurred in the 0900-1500 and 2100-0300, respectively, samples and are here considered to result from diurnal vertical migrations. The product, (cobalt content) X ($\Sigma\lambda$) X (wet weight/100 m^3),

gives the Co flux rate for each group of animals. The mean Co flux rate was multiplied by 24 hours to get the daily rate, 1.0 $\mu g/100\ m^3 \cdot day$ (Table 13). This is the turnover rate per m^2 in the upper 100 m depth attributable to zooplankton alone. Assuming that the migrating animals are below the thermocline twelve hours per day, we obtain the transport, 0.30 $\mu g/m^2 \cdot day$.

Kuenzler (1961) used the expression "participatory turnover time" to denote the turnover time of a substance in a system calculated from any one route by which this substance circulates, recognizing that this participatory turnover time would necessarily overestimate the true turnover time of the system. The concentration of dissolved cobalt given by Sillén (1961) was $10^{-7.9}$ to $10^{-8.8}$ M, which averages out to be approximately 0.4 μg Co/l or 40 $mg/100\ m^3$. The participatory turnover time for the upper 100 m depth calculated solely on the basis of the elimination rates of zooplankton is

$$\frac{40 \times 10^{-3}\ g/100\ m^3}{1 \times 10^{-6}\ g/100\ m^3 \cdot day} = 40 \times 10^3 \text{ days.}$$

Thus the zooplankton in the upper layers pass an amount of cobalt equal to that in the water through their bodies only once in about 100 years.

Several different factors act to shorten the turnover time of cobalt in the surface waters from our calculated value. As already shown, initial rates of elemental elimination are usually higher than the mean rates obtained from relatively long-duration experiments; one would expect animals in nature to cycle elements at more nearly the initial rates. Furthermore Odum (1961), Mishima and Odum (1963), and Rice (1963B) reported that beetles, snails, crabs, and clams eliminated Zn^{65} faster when released into their natural environment than while in laboratory containers; thus the elimination rates of our confined, unfed animals may be too low. Elements such as cobalt are also lost from plankton populations by pathways other than the elimination products that we measured. There are losses from moulted

exoskeletons, from production of gametes or young, and from mortality. Reid (1962, after Thrailkill, 1959) showed zooplankton concentrations more than 0.3 ml/l in the upper 140 m of large areas of the Eastern Pacific during a rich (cold) year. These values are ten or more times the zooplankton concentrations that we found; turnover rates would be correspondingly increased and turnover times shortened proportionately. Finally, turnover time of cobalt in the upper layers will be still further shortened in proportion to the cycling rates in phytoplankton, microflagellates, and nekton. Thus, we might guess that the 100 years estimate is high by a factor of ten or more.

Similarly, the turnover time of cobalt in the upper layers attributable to transport through the thermocline by migrating zooplankton is

$$\frac{40 \times 10^{-3} \text{ g/m}^2}{0.30 \times 10^{-6} \text{ g/m}^2 \cdot \text{day}} = 130 \times 10^3 \text{ days},$$

or about 350 years. The same factors discussed in the above paragraph will also increase transport through the thermocline by zooplankton. Odum (1961), Mishima and Odum (1963), and Rice (1963B), however, also reported that Zn^{65} is lost more slowly at low than at high temperatures; if animals experience this effect with cobalt in the colder water below the thermocline, then turnover times based on our experiments at surface temperatures would be proportionately too short. Other processes that contribute to transport through the thermocline, and therefore act to decrease this turnover time, are eddy diffusion, nekton migration, and the sinking of cobalt-containing particulate matter. Osterburg, Carey, and Curl (1963) suggested that rapid sinking of fecal pellets could account for the rapidity with which some radionuclides reach the ocean bottom, and we have seen that a moderate proportion of eliminated cobalt is particulate (Table 11). Besides fecal

material we may expect a rain of moulted exuviae and dead organisms. Using the above factors we might guess that turnover caused by transport through the thermocline is of the order 35-350 years.

Admittedly there are many uncertainties in the data on which the above turnover times for cobalt are based. However, there is even less quantitative data on the iodine, iron, manganese, and zinc content of important marine zooplankters, and their contribution to turnover of these elements cannot be estimated as was done for cobalt. The need for elemental analyses is demonstrated by the importance of Chaetognatha in the cobalt cycle. Assuming that the high cobalt content reported for Sagitta elegans (Nicholls, et al., 1959) is representative of all chaetognaths, this group, comprising 11% of the mean zooplankton mass, contributes 76% of the turnover in the upper 100 m. They comprise less than 5% of the migrating biomass, yet they carry 55% of the zooplankton-transported cobalt into deeper layers (Table 17).

SUMMARY

1. An evaluation was made of the importance of marine zooplankton in elemental cycling and transport of elements, especially cobalt, through the thermocline of the sea. Zooplankton density was measured in the eastern Pacific Ocean in spring 1962. Some measurements of elemental elimination rates were made using zooplankton labeled by fallout from a nuclear test; other measurements were made upon zooplankton labeled with tracer I^{131} or Co^{58}. The sea water in which the animals were kept during the excretion experiments was filtered and then passed through cation- and anion-exchange resins to learn the physical and chemical form of the eliminated isotopes.

2. Zooplankton hauls were taken vertically with a closing net from the depths 100-0 m, 300-100 m, and 500-300 m to determine the standing

crop and migration patterns for the most abundant animals. Over the whole day, the mean biomass (wet weight) in the upper 100 m was 3.87 g/100 m^3, of which 42% were copepods, 13% were pyrosomas, 13% were siphonophores, 11% were salps and doliolids, and 11% were chaetognaths. The diurnally migrating zooplankton amounted to 4.16 g/m^2, of which 22% were copepods, 19% were pyrosomas, 20% were siphonophores, 18% were salps and doliolids, 6.5% were medusae, and 4% were chaetognaths.

3. The rates at which elements were eliminated by zooplankton depended on the element, the animal species, and the time since they became labeled. In experiments performed soon after labeling, pteropods had elimination rates of 3-6%/hr for Mn, I, and Fe, 2-4%/hr for Co, and 1%/hr for Zn; pyrosomas had rates of 3-5%/hr for Mn and Fe, 2-3%/hr for Co, and 1%/hr, or less, for I and Zn; copepods had rates of 1-6%/hr for I and 4-6%/hr for Co; euphausiids eliminated Zn at rates of 3-8%/hr. Experiments performed at later times after labeling showed lower elimination rates.

4. Most of the iodine eliminated by zooplankton was probably I^-, but copepods also eliminated some cationic form. Most eliminated manganese was particulate, perhaps feces. Much of the eliminated iron was particulate, but significant amounts were also found to be soluble anionic (Pyrosoma, Salpa) or non-exchangeable (Sagitta). Significant amounts of eliminated cobalt were particulate, but most of it was cationic. Eliminated zinc also was frequently found in the particulate fraction, but anionic, cationic, and non-exchangeable zinc were also eliminated.

5. Using data from the literature on cobalt content of zooplankton and sea water and our values for cobalt flux rates of zooplankton, the turnover time for cobalt in the upper 100 m is not more than 100 years. Similarly, diurnally migrating zooplankton carry down and excrete below the thermocline a quantity of cobalt equal to that in the upper 60 m in not more than 350 years.

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Table 1. Wet weight (g/100 m^3) of all copepods (including Pleuromamma) at 3 depths throughout the day.

	Local Time			
Depth (m)	0300-0900	0900-1500	1500-2100	2100-0300
0-100 :	1.37	1.75	1.28	2.27
100-300 :	.72	.87	.61	.89
300-500 :	.68	.46	.44	.32

Table 2. Wet weights (g) and number of Pyrosoma verticillatum Neumann per 100 m^3 at three depths throughout the day.

	Local Time							
Depth (m)	0300-0900		0900-1500		1500-2100		2100-0300	
	Wt.	No.	Wt.	No.	Wt.	No.	Wt.	No.
0-100 :	1.02	13	0.29	10	0.22	10	0.55	9
100-300 :	0	0	0	0	0.03	1	0	0
300-500 :	0	0	0	0	0	0	0	0

Table 3. Wet weight (g/100 m^3) of Siphonophora at 3 depths throughout the day.

	Local Time			
Depth (m)	0300-0900	0900-1500	1500-2100	2100-0300
0-100 :	1.03	.20	.25	.52
100-300 :	.23	.11	.03	.12
300-500 :	.14	.28	.01	.09

Table 4. Wet weights (g/100 m^3) of medusae at 3 depths throughout the day.

	Local Time			
Depth (m)	0300-0900	0900-1500	1500-2100	2100-0300
0-100 :	.007	0	0	.28
100-300 :	.003	0	.006	.04
300-500 :	.15	.001	.03	0

Table 5. Wet weight (g/100 m^3) of ostracods and other small crustaceans at 3 depths throughout the day.

	Local Time			
Depth (m)	0300-0900	0900-1500	1500-2100	2100-0300
0-100 :	.046	.041	.096	.089
100-300 :	.068	.070	.041	.049
300-500 :	.021	.033	.028	.009

Table 6. Wet weights (g/100 m^3) of fish at 3 depths throughout the day.

	Local Time			
Depth (m)	0300-0900	0900-1500	1500-2100	2100-0300
0-100 :	.029	.002	.034	.063
100-300 :	.002	.007	0	.011
300-500 :	.13	.003	.073	.058

Table 7. Gross gamma activity (10^3 cpm/g wet weight) in zooplankton samples taken over an eleven day period following the nuclear test.

Day:	1	4	4	5	7	8	10	11
Depth (m):	10	30	300	150	25	25	20	25
Local time:	2330	0100	0600	0545	2240	2215	0410	0325
Pteropoda	400	270				48		51
Heteropoda								1.7
Pyrosoma	6.7	5.4	1.4	0.22	3.9	0.73	1.7	.86
Salpidae						0.73	5.6	1.1
Euphausiidae	45	70	14	49		0.50	13	6.0
Chaetognatha		8.6	2.4			1.4	2.3	
Ctenophore (Berŏe cucumis Fabricius)	0.51	0.54		8.1			2.5	1.9
Mixed plankton	22		41	14				
Copepoda (Candacia ethiopica (Dana))			75					
Amphipoda	1.5	22	9.0	3.9				
Decapoda larvae	38					9.7		
Squid		13				6.2		
Medusae						26		
Fish						23		

Table 8. Radioactivity of zooplankton during the 11 day experimental period. Activities of all isotopes are corrected for decay to 13 June 1962.

	Day	n	cpm/g Wet Weight				
			I^{131}	Zn^{65}	Fe^{55}	Mn^{54}	Co ^{58,60}
Pteropoda	1	3	12,000	610	660	56	1,450
	4	1	5,700	340	230	28	260
	8	1	320	170	54		
	11	1	140	390		5.6	
Heteropoda	11	1		53	15		
Pyrosoma	1	1	230	77			
	4	3	92	29	5.8	0.7	17
	7	1		67			
	8		9.4	11			
Salpidae	10	1		18	14		
	11	1		6	4.8		
Euphausiidae	1	1		76			
	4	2		226			
	11	1		170			
Chaetognatha	8	1		24	9.7		
	10	1		40	11		
Ctenophora	1	1	52				
Mixed plankton	1	1	1,000	200	44		
	6	1	310				
Copepoda	4	1		1300			

Table 9. Elimination rates of I^{131} by zooplankton; n is the number of replication, ΔT is the duration of the experiments.

Species	n	ΔT (hr)	$\bar{\lambda}$ = Mean Elimination Rate (hr^{-1}) MF	SCE_1	SAE_1	WAE_1	$Effluent_1$	Total
Pteropoda								
Cavolinia inflexa	1	10	-	-	.032	-	.017	.049
C. inflexa plus	3	10-16	-	-	.030	-	.020	.050
Euclio pyramidata	1	10	-	-	-	-	.005	.005
Pyrosomatidae								
Pyrosoma verticillatum	3	13-18	-	-	0	-	.007	.007
Ctenophora								
Beroe cucumis	1	18	-	-	-	-	.015	.015
Mixed plankton	2	13-24	-	-	-	-	.014	.014
Copepoda								
Neocalanus gracilis (Dana)* (13hr)	2	T_o-T_1=6	.001	.003	.008	0	0	.012
	2	T_1-T_2=6	0	.001	.001	0	0	.002
Pleuromamma xiphias* (22 hr)	2	T_o-T_1=4	.001	.009	.048	0	0	.058
	2	T_1-T_2=7	.001	.004	.005	0	0	.010
P. abdominalis* (11 hr)	3	T_o-T_1=5-9	.001	.004	.016	0	0	.021
	3	T_1-T_2=4-6	0	.001	.003	0	0	.004

*Experiments performed aboard ANTON BRUUN. Hours of isotope uptake are given in parentheses.

Table 10. Elimination rates of Mn^{54} by zooplankton; n is the number of replications, ΔT is the duration of the excretion experiments.

Species	n	ΔT (hr)	$\bar{\lambda}$ = Mean Elimination Rate (hr^{-1})					
			MF	SCE_1	SAE_1	WAE_1	$Effluent_1$	Total
Pteropoda								
Cavolinia inflexa	1	10	.007	0	0	0	0	.007
C. inflexa plus *Euclio pyramidata*	2	10	.040	0		.003	0	.043
Pyrosomatidae								
Pyrosoma verticillatum	2	16	.039	.013		0	0	.052

Table 11. Elimination rates of $Co^{58, 60}$ by zooplankton; n is the number of replications. ΔT is the elimination period; in parentheses are the isotope uptake periods aboard ANTON BRUUN*.

Species	n	ΔT (hr)	$\bar{\lambda}$ = Mean Elimination Rate (hr^{-1}) MF	SCE_1	SAE_1	WAE_1	$Effluent_1$	Total
Pteropoda								
Cavolinia inflexa	1	10	.009	0	0	0	0	.009
C. inflexa plus Euclio pyramidata	3	9-15		0			0	.032
C. inflexa* (33 hr)	2	20	.003	.007	.001	0	.007	.018
Cuvierina columnella* (33 hr)	2	7-12	.004	.001	0	0	.010	.015
Pyrosomatidae								
P. verticillatum	2	16	0	.017		0	0	.017
P. verticillatum* (11 hr)	2	T_o-T_1=10	.006	.011	.001	0	.014	.032
	2	T_1-T_2=10	.008	.022	.002	0	.027	.059
Copepods								
Neocalanus gracilis* (12-15 hr)	2	T_o-T_1=4-5	.007	.022	0	.001	.027	.057
	1	T_1-T_2=6-7	.003	.007	.001	0	.009	.020
Pleuromamma xiphias* (12-22 hr)	3	T_o-T_1=7-10	.012	.012	0	0	.019	.043
	2	T_1-T_2=9	.003	.009	0	0	.012	.024
P. abdominalis* (12 hr)	2	T_o-T_1=10	.030	.014	.001	0	.014	.059
	2	T_1-T_2=9	.006	.010	.002	0	0	.018

Table 12. Elimination of Fe^{55} by zooplankton; n is the number of replications, ΔT is the duration of the excretion experiments.

Species	n	ΔT (hr.)	$\bar{\lambda}$ = Mean Elimination Rate (hr^{-1}) MF	SCE_1	SAE_1	WAE_1	Effluent	Total
Pteropoda								
Cavolinia inflexa plus Euclio pyramidata	3	10-16	.021	.017		-	.009	.047
Pyrosomatidae								
Pyrosoma verticillatum	2	16	.006	.021		-	.006	.033
	1	13	0	0	.018	0	0	.018
Heteropoda								
Carinaria lamarcki Péron & Lesueur	1	24	.012	0	0	0	0	.012
Salpidae								
Salpa fusiformis	1	15	.031	0	.011	-	.041	.083
Cyclosalpa pinnata (Forskål)	1	23	.036	0	0	-	0	.036
Chaetognatha								
Sagitta hexaptera	1	15-18	0	0	0	-	.010	.010

Table 13. Elimination rates of Zn^{65} by zooplankton; n is the number of replications, ΔT is the duration of the excretion experiments.

Species	n	ΔT (hr)	$\bar{\lambda}$ = Mean Elimination Rate (hr^{-1})					
			MF	SCE_1	SAE_1	WAE_1	$Effluent_1$	Total
Pteropoda								
Cavolinia inflexa	1	10	.005	.001	.003	.001	.004	.014
C. inflexa plus	3	10	.003	.006		0	.002	.011
Euclio pyramidata	2	18-26	0	.002	.003	.001	0	.006
Pyrasomatidae	2	15	.003	.007		.001	0	.011
Pyrosoma verticillatum	4	13-18	.003	.002	.002	.001	.001	.009
Mixed plankton	1	13	0	.003	.002	.001	0	.006
Copepoda								
Candacia ethiopica	1	13	.006	0	0	0	.006	.012
Euphausiidae								
Mixed species	4	9-25	.015	.006	.012	.004	.004	.041
Salpidae								
Salpa fusiformis	1	15	.010	.011	.026	-	0	.047
Cyclosalpa pinnata	1	23	.004	.002	.008	-	0	.014
Chaetognatha								
Sagitta hexaptera	2	15-18	.002	.012	.007	-	.010	.031
Heteropoda								
Carinaria lamarcki	1	24	.001	.001	.001	-	0	.003

Table 14. Elimination rates ($\sum \lambda$) of isotopes from zooplankton aboard ANTON BRUUN (T_o-T_1 only) and over the 11 day experimental period aboard PIONEER. The values are mean loss rates by all pathways - particulate, exchangeable, and non exchangeable.

			PIONEER (day)						
	Isotope	ANTON BRUUN	2	4	5	7	9	10	11
Pteropoda	Mn^{54}		.049	.038					
	I^{131}		.055	.033			.005		0
	Fe^{55}		.048	.042			0		
	$Co^{58,60}$	.016	.028	.040					
	Zn^{65}		.013	.009			.009		.002
Pyrosoma verticillatum	Mn^{54}			.052					
	I^{131}		.007	.008					
	Fe^{55}			.033	.018				
	Co^{58}	.032		.017					
	Zn^{65}		.005	.011		.004	.006		
Sagitta hexaptera	Zn^{65}						.029	.035	
Euphausiidae	Zn^{65}		.084	.033					.017
Salpidae	Fe^{55}						.083	.036	
	Zn^{65}						.047	.014	
Neocalanus gracilis	I^{131}	.012							
	Co^{58}	.057							
Pleuromamma xiphias	I^{131}	.058							
	Co^{58}	.043							
P. abdominalis	I^{131}	.021							
	Co^{58}	.059							

Table 15. Turnover times of elements in small crustaceans. Values marked by asterisk are calculated from data given by the various authors.

Organism	Element	Turnover Time (days)	Remarks	Reference
Calanus finmarchicus	P	0.5	fast reaction	Conover, 1961
" "	P	19	slow reaction	" "
" "	P	20		Marshall and Orr, 1961
Daphnia magna	P	~ 2.6		Rigler, 1961
Daphnia pulex	Fe	< 10*	assuming Fe excretion proportional to hemoglobin breakdown (Smaridge, 1956).	Green, 1955
Artemia	P	0.6		Harris, 1957
"	Co	~ 3*	either ingested or absorbed radiocobalt	Rice, 1963 A
Artemia	Zn	0.9*	ingested Zn^{65}; first 2 days	Rice, 1963 B
"	"	1.4*	" " ; next 8 days	" " "
"	"	0.7*	absorbed Zn^{65}; first 2 days	" " "
"	"	1.7*	" " ; next 8 days	" " "
Idothea baltica	Zn	1.5*	ingested Zn^{65}, fast reaction	Odum and Golley, 1963
" "	Zn	23*	" " slow reaction	" " " "
" "	Zn	0.6*	absorbed Zn^{65}, fast reaction	" " " "
	Zn	22*	absorbed Zn^{65}, slow reaction	" " " "
Gammarus locusta	P	1.8		Harris, 1957
Lembos intermedius	P	1.7		Johannes, 1964

Table 16. Relative adsorption on SCE resin (Dowex AG 50W-8; 0.5N HCl; Strelow 1960), and the probable form and concentration in sea water (Sillén 1961) of seven cations.

Relative Adsorption		In Sea Water	
Ion	Adsorption	Probable Form	Concentration (-log M)
Mn^{++}	84	$Mn(OH)_{3,4}?$	6.7-7.9
Co^{++}	72	Co^{++}; $CoSO_4$(5-10%)	7.9-8.8
Fe^{+++}	225	$Fe(OH)_3?$	6.0-7.9
Zn^{++}	64	^{+}Zn	6.5-7.7
Na^{+}	12	Na^{+}	0.33
Mg^{++}	88	Mg^{++}	1.27
Ca^{++}	151	Ca^{++}	1.99

Table 17. Contribution of zooplankton to cobalt turnover in the upper 100 m, and to its transport through the thermocline. Weights and Co content are based on wet weight.

Zooplankton Group	Co		Weight/m^2 in upper 100 m		Co flux	
	Content (ppm)	$\sum\lambda$ (hr^{-1})	Mean	Max.-Min.	Mean	Max.-Min.
			($g/100\ m^3$)		($\mu g/100\ m^3 \cdot hr$)	
1. Copepoda	.054	.057	1.64	.92	.0051	.0028
2. Pyrosoma	.03	.036	.52	.80	.0006	.0009
3. Siphonophora	.05	(.03)	.50	.83	.0008	.0012
4. Salpidae-Doliolidae	.031	(.03)	.44	.76	.0004	.0007
5. Chaetognatha	2.5	(.03)	.43	.18	.032	.014
6. Euphausiidae	.19	(.03)	.10	.13	.0006	.0007
7. Medusae	.087	(.03)	.072	.28	.0002	.0007
8. Ostracoda, other crust.	.12	(.03)	.068	.055	.0002	.0002
9. Pleuromamma	.14	.049	.026	.072	.0002	.0005
10. Pteropoda	2.4	.021	.038	.07	.0019	.0035
11. Fish	.1	(.03	.032	.061	.0001	.0002
Total			3.870	4.156	.0421	.0254
Hours per day					24	12
Daily Co flux ($\mu g/100m^3 \cdot day$)					1.0	0.30

FIGURE LEGENDS

Figure 1. Apparatus for removing I^{131} from tissues of zooplankton and recovering it for counting.

Figure 2. Vertical distribution of Salpidae and Doliolidae at four periods of the day.

Figure 3. Vertical distribution of Chaetognatha at four periods of the day.

Figure 4. Vertical distribution of Euphausiidae at four periods of the day.

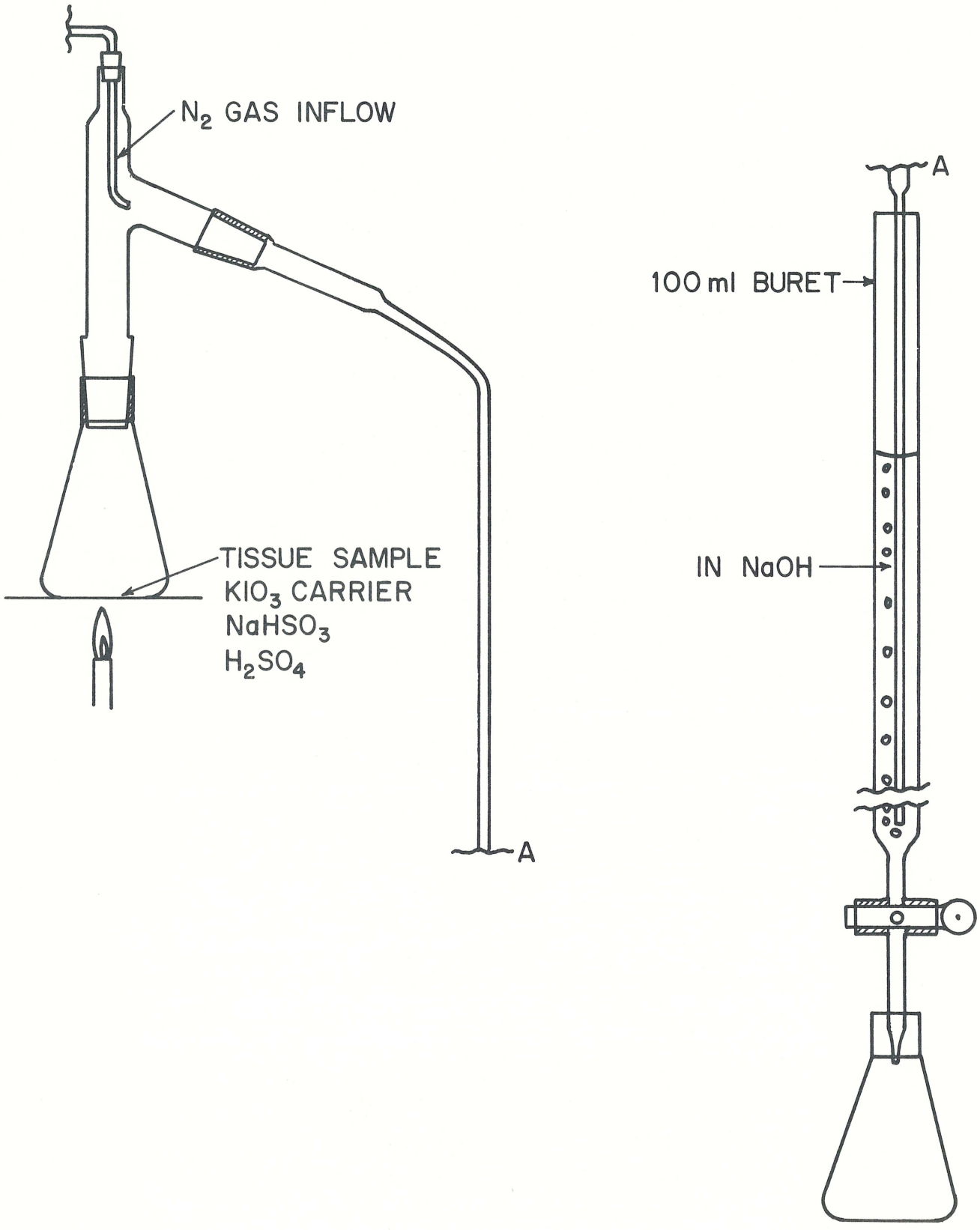
Figure 5. Vertical distribution of Pleuromamma spp. at four periods of the day.

Figure 6. Vertical distribution of Pteropoda at four periods of the day.

Figure 7. Breakthrough of I^{131} (as IO_3^- and I^-) in sea water on SCE (Rexyn Ag-50, Na^+ form) and SAE (Rexyn AG-1, Cl^- form) resins.

Figure 8. Breakthrough of divalent Mn^{54} and Co^{58} in sea water on SCE (sea water equilibrated) and SAE (Cl^- form) resins.

Figure 9. Breakthrough of trivalent Fe^{59} and divalent Zn^{65} in sea water on SCE (sea water equilibrated) and SAE (sea water and Cl^- form, respectively) resins.



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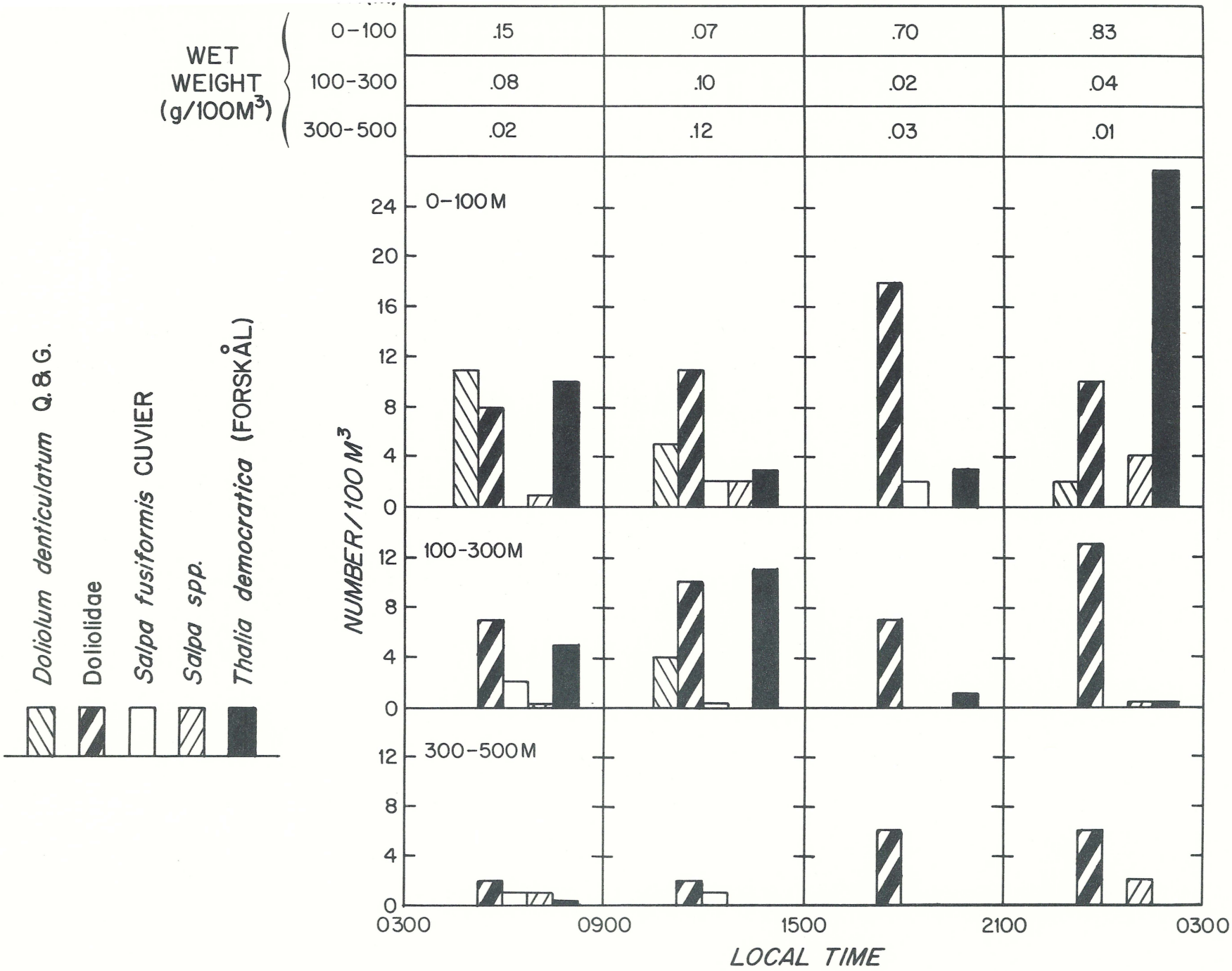


Figure 2. Vertical distribution of Salpidae and Doliolidae at four periods of the day.

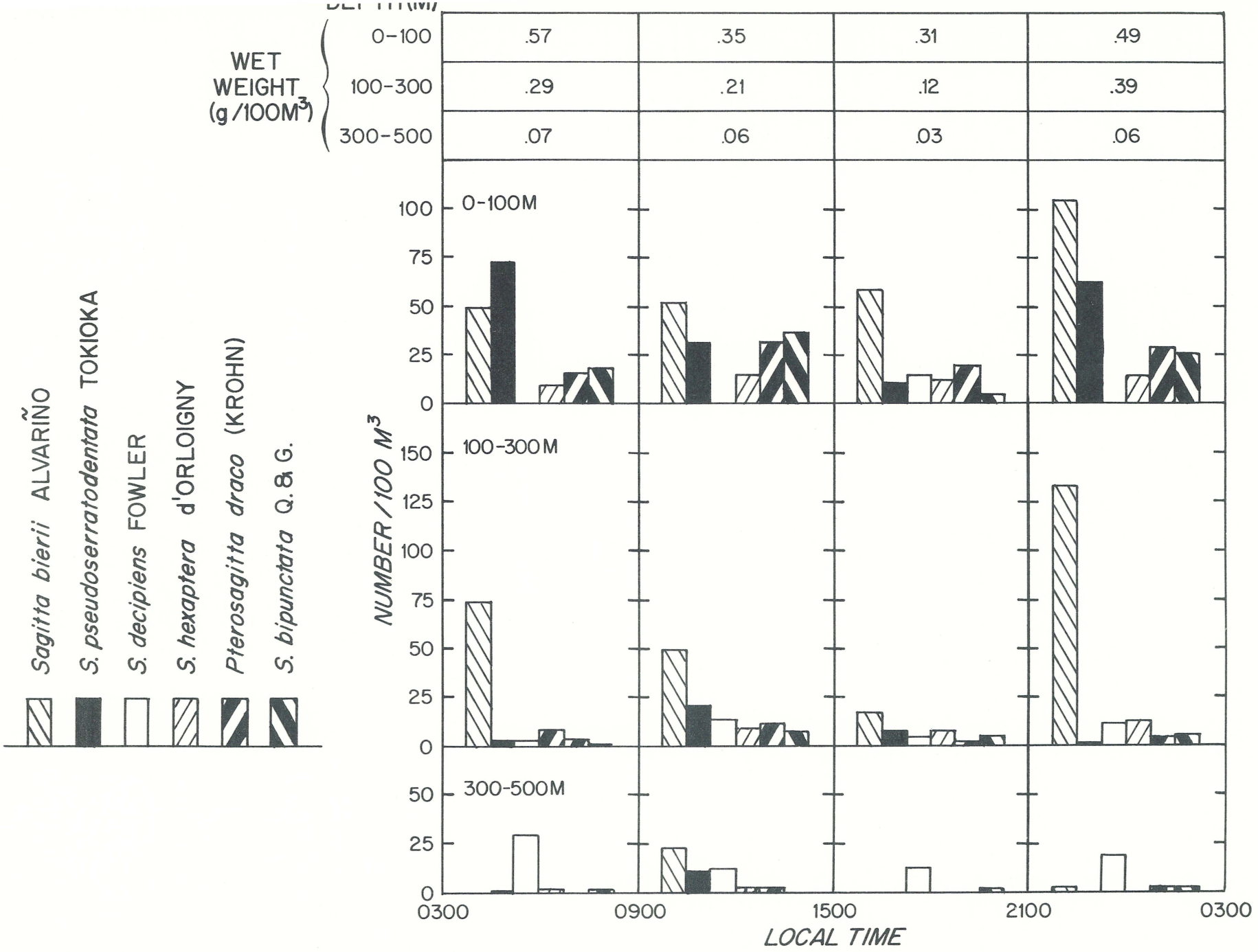


Figure 3. Vertical distribution of Chaetognatha at four periods of the day.

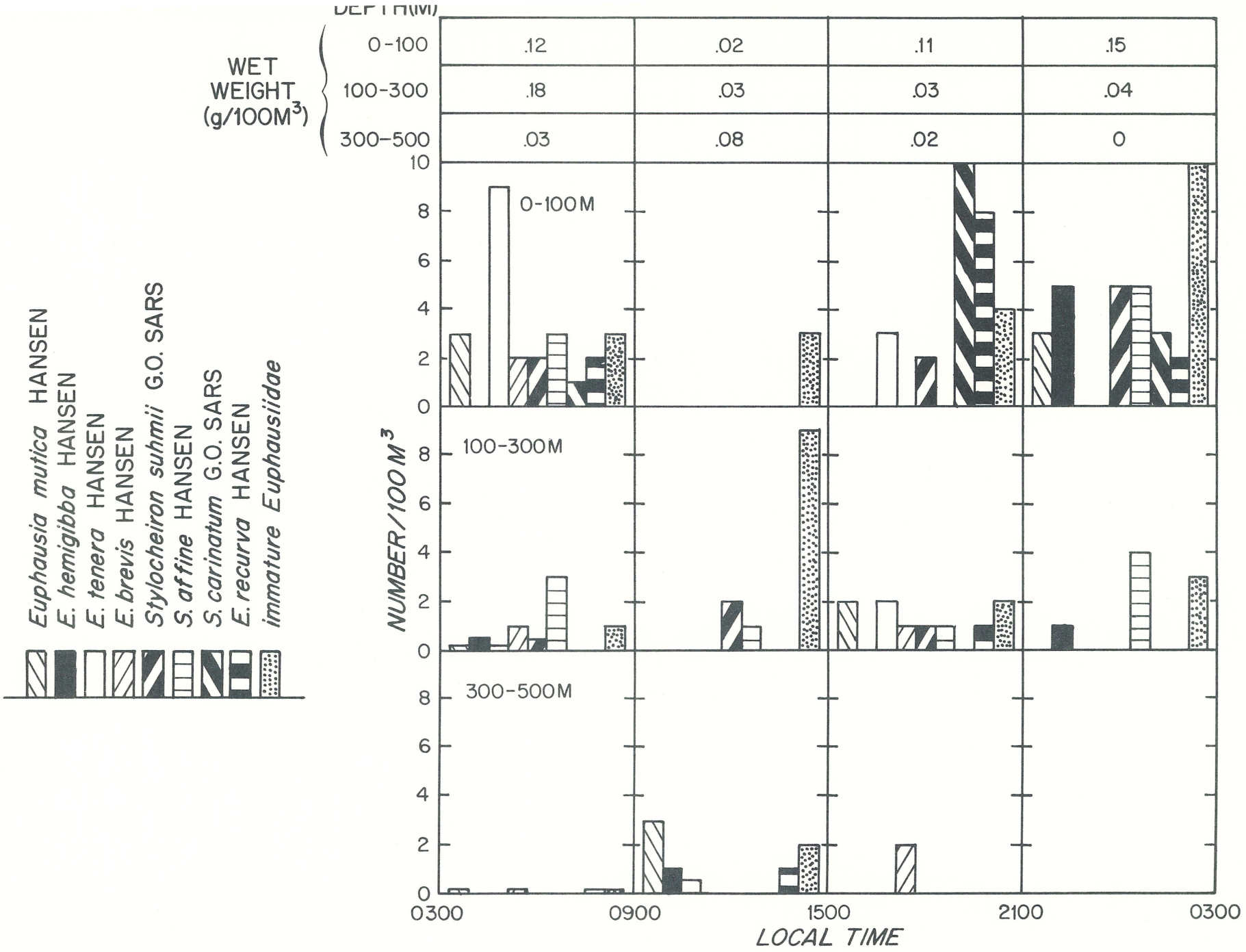


Figure 4. Vertical distribution of Euphausiidae at four periods of the day.

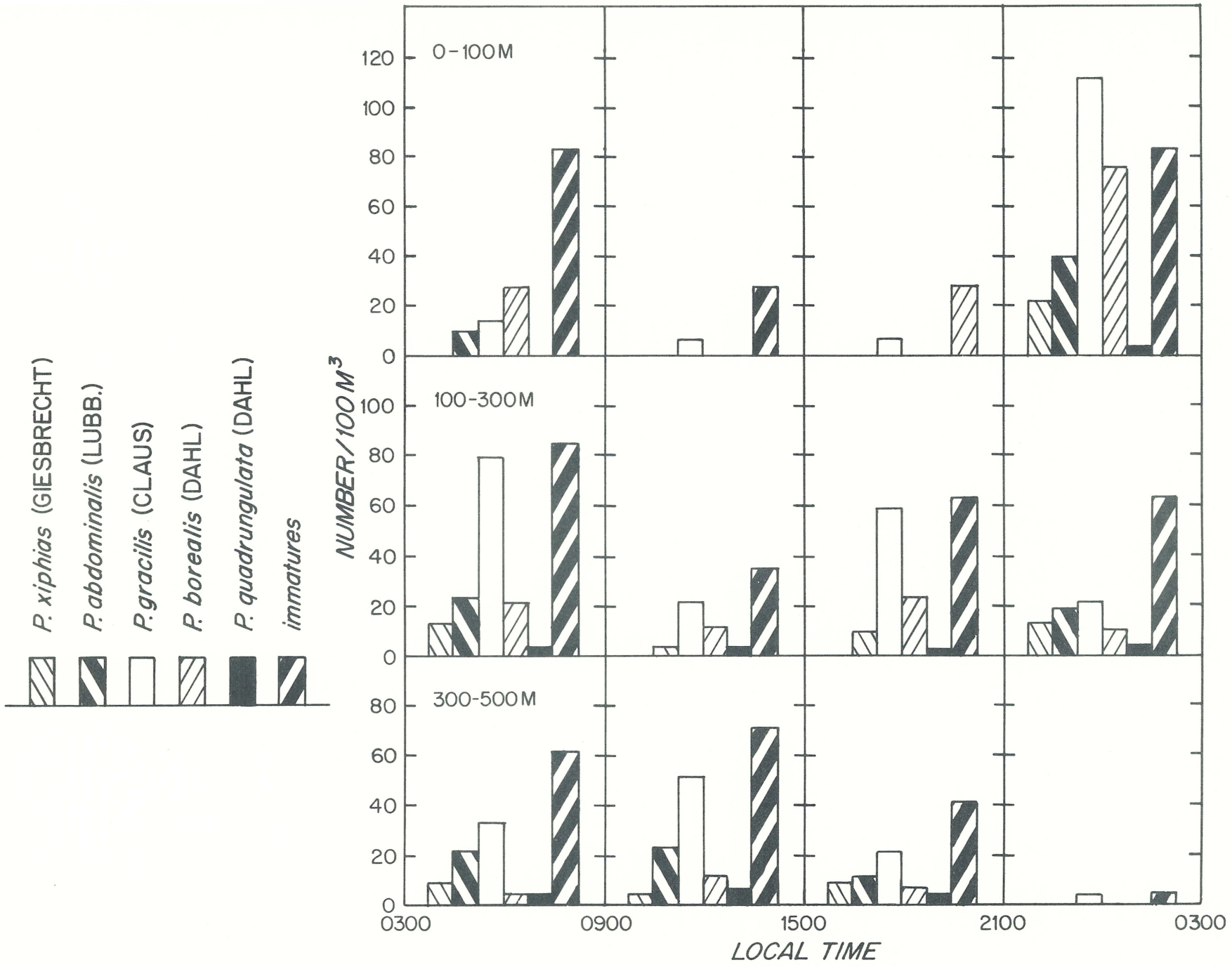


Figure 5. Vertical distribution of Pleuromamma spp. at four periods of the day.

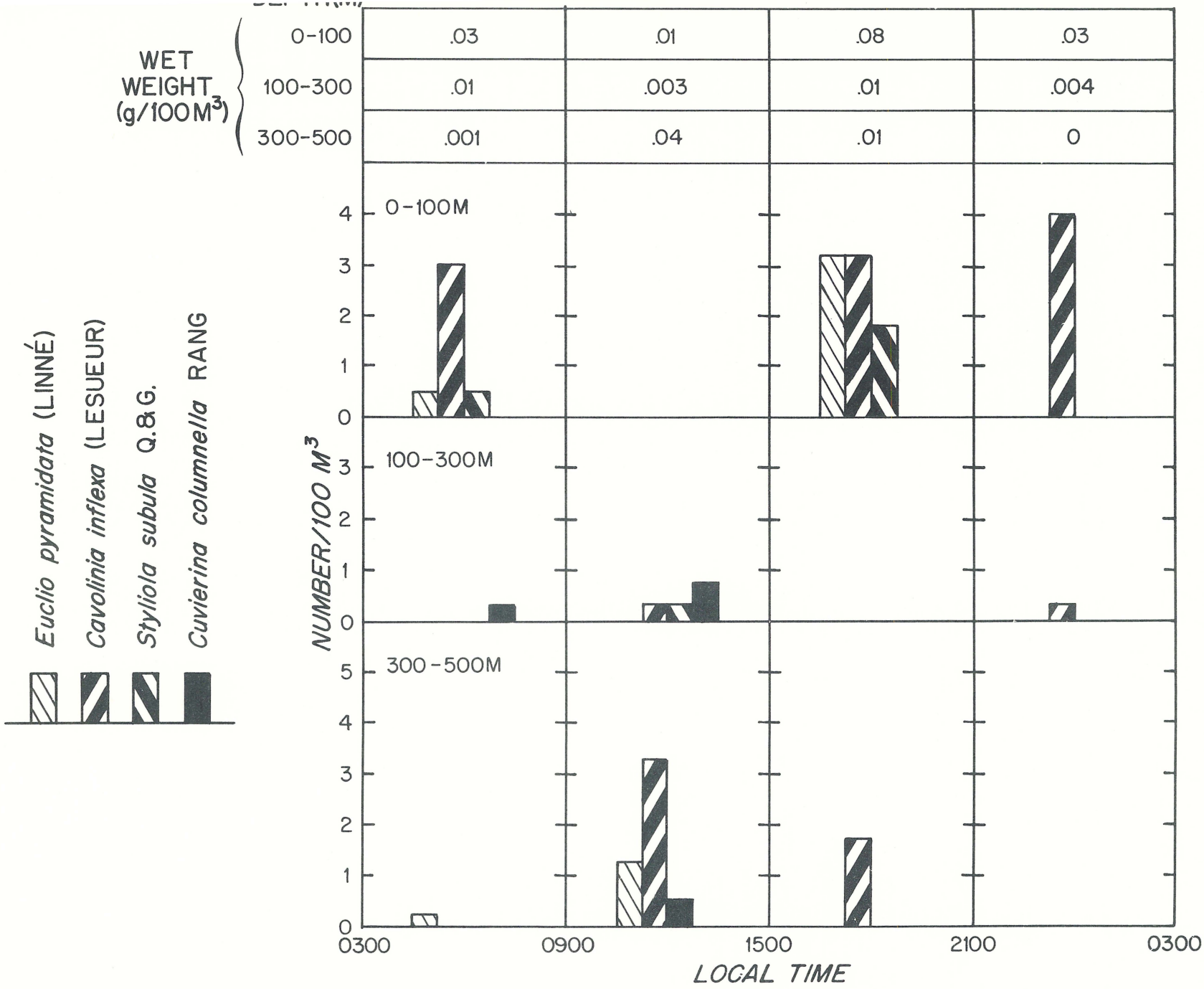
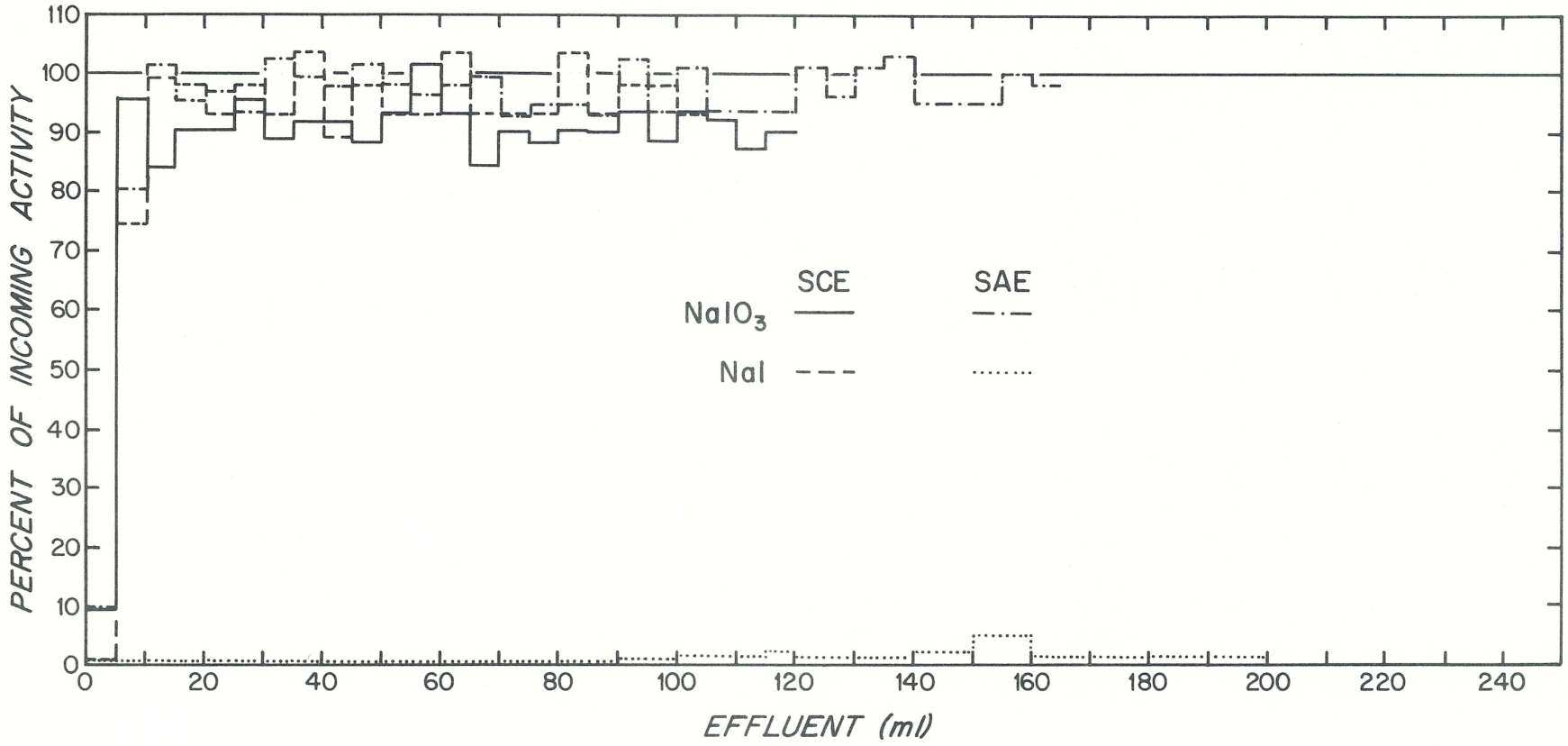


Figure 6. Vertical distribution of Pteropoda at four periods of the day.



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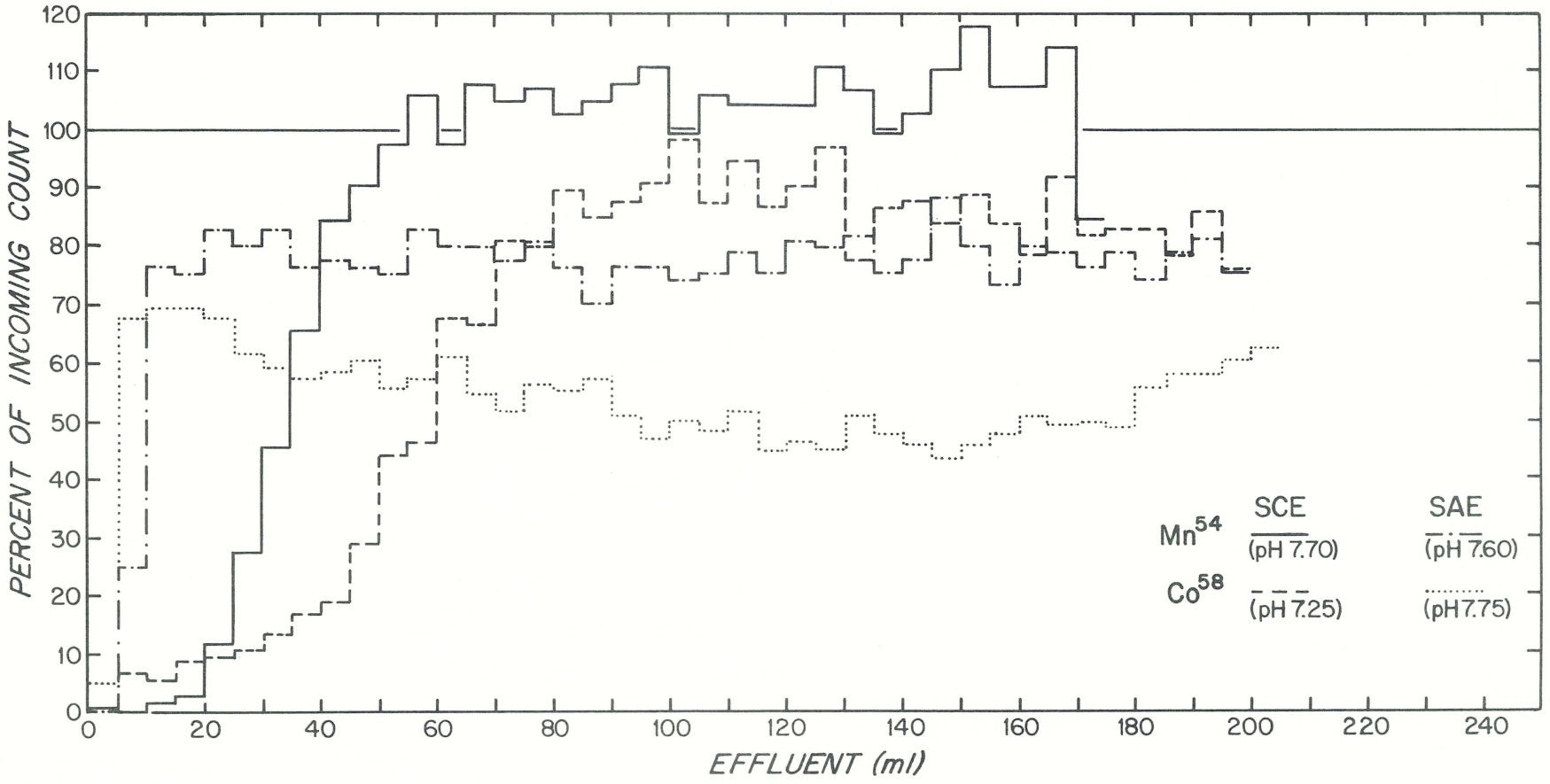

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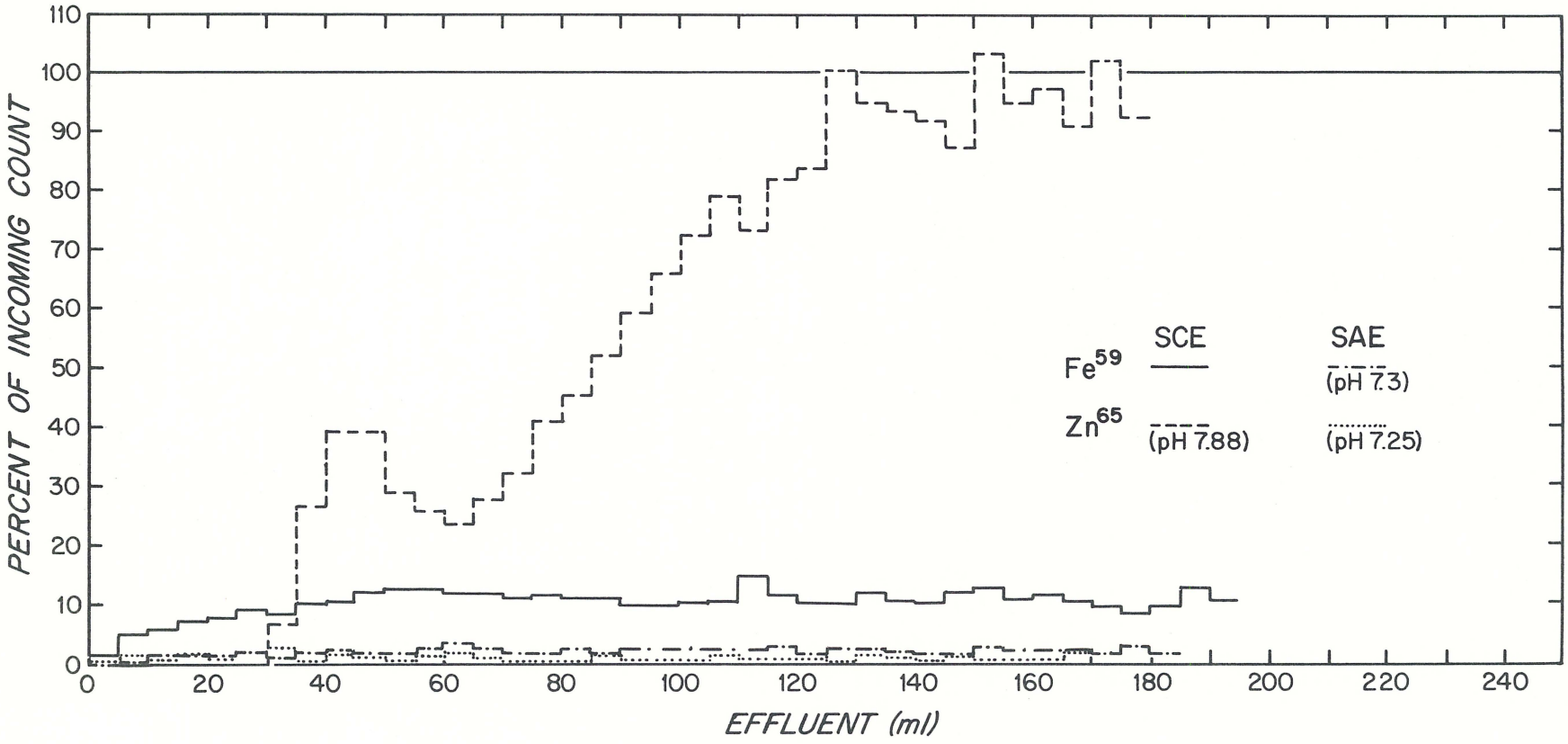

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Zooplankton Distribution and Isotope Turnover
During Operation SWORDFISH

Unclassified

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